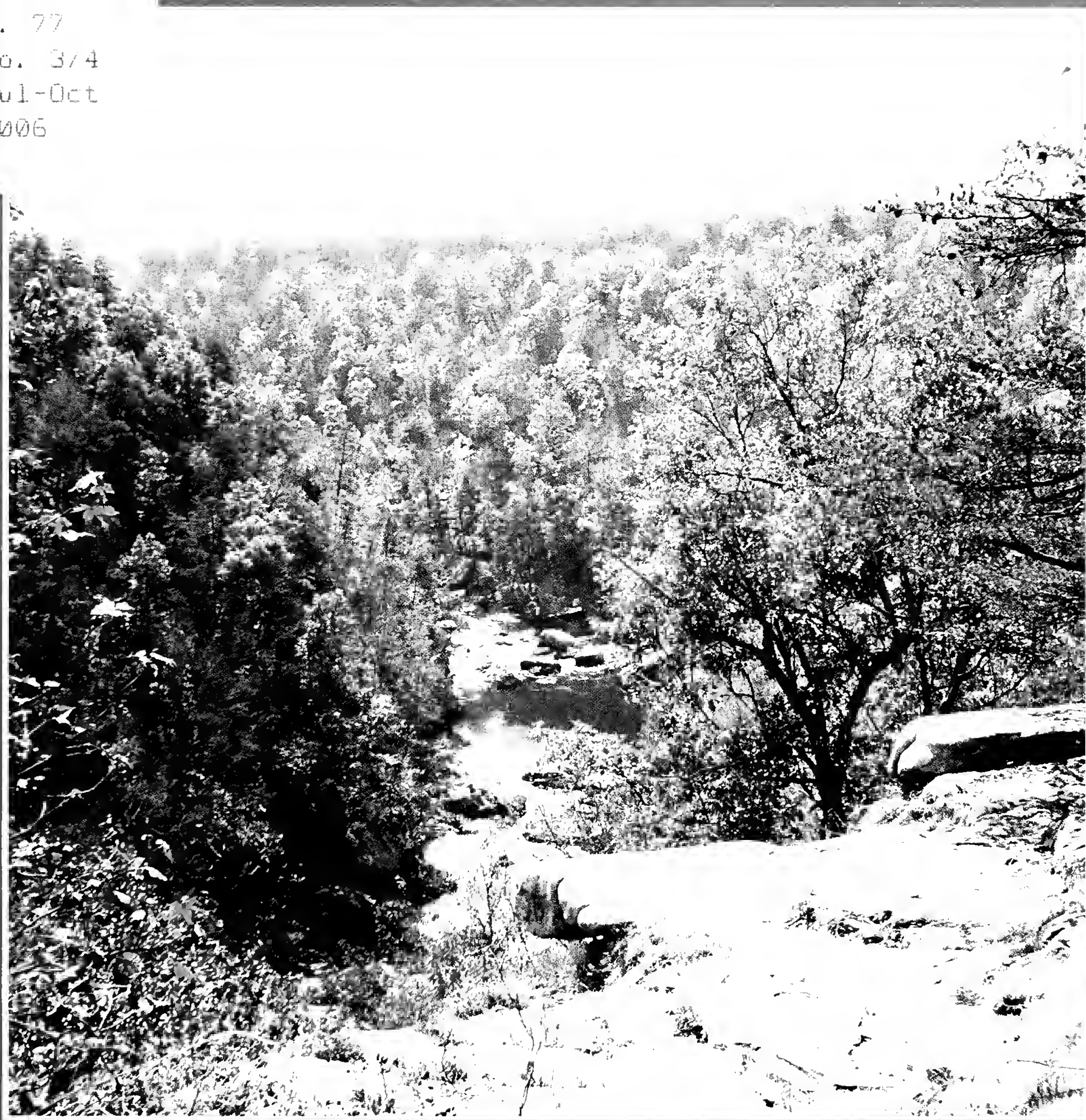
THE JOURNAL OF THE ALABAMA ACADEMY OF SCIENCE



VOLUME 77 JULY/OCTOBER 2006 NO. 3-4

Cover Photograph: Autumn Leaves of Little River Canyon (Fort Payne, Alabama).

Photographer: David A. Francko, Assistant Vice President for Academic Affairs and Dean of the Graduate School, and Professor of Biology, The University of Alabama.

Editorial Comment:

On behalf of the Alabama Academy of Science, I would like to express my gratitude and appreciation to Mrs. Sue Bradley for her many years of contributions to the Alabama Academy of Science Journal. Mrs. Bradley served for many years as an assistant to the editor, overseeing the layout of the journal, article organization and journal mailing. She is indeed someone to respect and depend on to get the job done. We wish her the best in her future endeavors.

Safaa Al-Hamdani
Editor, Alabama Academy of Science Journal

THE JOURNAL OF THE ALABAMA ACADEMY OF SCIENCE AFFILIATED WITH THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

VOLUME 77 JULY/OCTOBER 2006 NO.3-4

Publication and Subscription Policies:
Submit all manuscripts and pertinent correspondence to the Editor. Each manuscript will receive at least two simultaneous reviews. For style details, follow instructions to Authors (see inside back cover).

Reprints requests must be addressed to Authors.

Subscriptions and Journal Exchanges: Address all Correspondence to the Chairman of the Editorial Board.

ISSN 002-4112

BENEFACTORS OF THE
JOURNAL OF THE
ALABAMA ACADEMY OF SCIENCE

The following have provided financial support
to partially defray publication costs of the journal.

AUBURN UNIVERSITY
AUBURN UNIVERSITY AT MONTGOMERY
BIRMINGHAM-SOUTHERN COLLEGE
JACKSONVILLE STATE UNIVERSITY
SAMFORD UNIVERSITY
TROY STATE UNIVERSITY
TUSKEGEE UNIVERSITY
UNIVERSITY OF ALABAMA
UNIVERSITY OF ALABAMA AT BIRMINGHAM
UNIVERSITY OF MONTEVALLO
UNIVERSITY OF NORTH ALABAMA
UNIVERSITY OF SOUTH ALABAMA

CONTENTS

ARTICLES:

BOOK REVIEW:

PLASMID PROFILE OF ANTIBIOTIC RESISTANT *Escherichia coli* ISOLATED FROM CHICKEN INTESTINES

Saif N. Al-Bahry, Basma M. Al-Mashani, Abdulkadir E. Elshafie, N. Pathare and Asila H. Al-Harthy
Department of Biology, College of Science, Sultan Qaboos University. P.O.Box 36, Al-Khodh, P.C. 123, Sultanate of Oman.

Correspondence: Al-Bahry, Saif (snbahry@squ.edu.om)

ABSTRACT

Fifty-seven strains of *Escherichia coli* from chicken intestines were isolated and tested against 15 antibiotics by the disk diffusion method. All strains were resistant to at least one of the tested antibiotics. Resistance to 12 antibiotics was the maximum observed. 26.3% of the isolates exhibited multiple resistances to 50% of the tested antibiotics. Most of the isolates were resistant to tetracycline and nalidixic acid. R-plasmids were extracted and separated by agarose gel electrophoresis for profiling. Plasmid profiling of antibiotic resistant *Escherichia coli* isolates revealed that the isolates contained various size R-plasmids. Although some strains exhibited different antibiotic resistance patterns, some of their plasmids had similar migration patterns on agarose gel electrophoresis. Multiple resistances are conferred by R-plasmids of different sizes. The high prevalence of antibiotic resistance conferring plasmids observed in this study may be due to the increasing widespread use of antibiotics.

INTRODUCTION

Antibiotics have helped in reducing the diseases in the poultry farms. However, there is a growing awareness of public health concerns associated with the use of antibiotics (Rice *et al.*., 1995). Although antibiotic use is under national regulations of Oman, farmers still overuse antibiotics. Carraminana (et al.., 2004) reported that 40% of the antibiotics produced in the United States were used in stock feeds. The widespread use of various antibiotics for treating chicken infections has created for antibiotic resistant bacterial strains (Chee-Sanford et al.., 2001, Sackey et al.., 2001). Bacterial isolates obtained by Carraminana et al.. (2004) from a poultry slaughter house in Spain had high percentages of resistance to many antibiotics such as neomycin (53.4%), tetracycline (21.8%), and streptomycin (11.3%). Saenz et al.. (2000) reported a high frequency of resistance to ampicillin (65.7%), gentamicin (22.2%), and amikacin (21.6%) in bacterial strains isolated from animals.

Multiple antibiotic resistant strains can be transported from animals to humans by food (Cordano and Virgilio, 1996). Linton et al.. (1977) reported that multiple resistant bacterial strains were transmitted to humans by raw meat and milk. A poultry feces is a

potential source of antibiotic resistant bacteria. If released into the environment, resistant strains may contaminate water and food sources and can be a potential threat to human health (Chee-Sanford et al.., 2001). *Escherichia coli* strains isolated from sewage treatment plants were reported to be resistant to various antibiotics (Osterblad et al.., 2000 and Reinthaler et al., 2003).

In Saudi Arabia, *Escherichta coli* isolated from chicken intestines were found to be resistant to many antibiotics such as ampicillin, chloramphenicol, gentamycin, tetracycline, trimethoprim and sulphamethoxazole (Al-Ghamdi et al.., 1999). Mirza et al.., (2000) reported that antimicrobial resistance was transferable from *Salmonella* spp to *Escherichia coli* as well as between other members of the intestinal normal flora.

Plasmids are a major mechanism for the spread of antibiotic resistant genes in bacterial populations (Smalla et al.., 2000). Conjugation occurs by F- plasmids that can transfer genes encoded for multiple resistance and mobilize other non-conjugative plasmids to host cells (Saxena et al., 1984). Multiple resistance genes are harbored on R-plasmids some of which are conjugative (Elwell and Falkows, 1980). *Escherichia coli* has been reported to transfer the antibiotic resistant genes to enteric pathogenic and normal flora bacteria such as *Salmonella* spp and *Proteus* spp (Platt et al.., 1986). The objective of this study was to investigate plasmid profile of antibiotic resistant *Escherichia coli* isolated from chicken intestines.

MATERIALS AND METHODS

Twenty-eight chicken intestines were collected from different local slaughter houses. *Escherichia coli* strains were isolated according to standard methods (Sonnenwirth and Jarett, 1980). The isolates were identified biochemically by API system (API Analytical Products, New York, NY). Forty-seven *Escherichia coli* isolates were tested for susceptibility to antibiotics following the disk diffusion method (Bauer *et al..*, 1966). Fifteen antibiotics were used: Amikacin (AK) 30 μg, Ampicillin (AMP) 10 μg, Carbenicillin (CB) 100 μg, Cephotaxin (CTX) 30 μg, Chloramphenicol (CM) 30 μg, Gentamycin (GM) 10 μg, Kanamycin (KM) 30 μg, Minocylin (MH) 30 μg, NAlidixic Acid (NA) 30 μg, Neomycin (NM) 30 μg, Sulphamethoxazole (SMX) 30 μg, Streptomycin (SM) 10 μg, Tetracycline (TE) 30 μg, Tobramycin (TOB) 10 μg, and Trimethoprim (TMP) 5 μg. Inhibition zone diameters were measured after 24 hours of incubation at 37°C. Antibiotic resistance patterns were recorded. A standard *Escherichia coli* (ATCC10536) was used as a control.

Plasmids were extracted using a High Pure Plasmid Isolation Kit (Roche, 2003). The extracted plasmids were separated by agarose gel electrophoresis for their profiling. Gel electrophoresis was carried out on 0.7% agarose grade gel at 100 v for 2h (Sambrook et al, 1989).

RESULTS

Forty-seven *Escherichia coli* strains were isolated from twenty-eight chicken intestines and tested against 15 antibiotics. All strains were resistant to at least one antibiotic (Table 1; Fig. 1). Nineteen percent of the isolates (9 out of 47 isolates) were resistant to four tested antibiotics. Forty-five *Escherichia coli* isolates were resistant to 5-7 antibiotics. The broadest range of drug resistance was up to 12 antibiotics (2.1%).

Table 1: Antibiotic resistance patterns and plasmid contents of *Escherichia coli* isolates.

Isolate number	Antibiotic resistance pattern	No of antibiotics *E. coli* isolates resistant	No of plasmids	Plasmid m.w. (K.b)
1	KMNMSMTE	4	3	66,48,22
2	KMNANMSMTE	5	4	61,51,29,14.5
3	KMNANMTE	4	2	26,11
4	KMNANMSMTE	5	4	66,48,22,8.8
5	KMNANMTE	4	4	66,48,26,8.8
6	GMKMNANMSMTE	6	1	51
7	KMNANMSMTE	5	1	51
8	CMNASMTE	4	1	66
9	TE	1	2	58,26
10	CMMHNASMXSMTETMP	7	3	31,10.5,2.9
11	AMPCBCMKMMHNASMXTETMP	9	4	61,12.7,2.3.3
12	AMPCBNASMXSMTETMP	7	1	3.3
13	CMMHNASMXSMTETMP	7	2	61,31
14	KMNMSMTE	4	1	6
15	AMPCBCMKMMHNASMXTETOBTMP	10	3	45,28,11.5
16	KMMHNASMXTETOBTMP	7	3	45,28,11.5
17	NASMXTETMP	4	2	58,43
18	AMPCBNATE	4	1	14.5
19	AMPCBCMKMNANMSMXSMTETMP	10	4	66,40,16.5,6.3
20	SMTETOB	3	1	40
21	KMNMTE	3	3	45,16.5,8.2
22	CMKMNANMSMTE	6	2	54.11.5
23	AMPCBKMNANMSMXSMTETMP	9	1	43
24	NASMXSMTETMP	5	2	51,12.5
25	KMNMSMTE	4	5	45,38,26,22,6.8
26	NASMTE	3	5	54,38,26,12,3.8
27	KMMHNANMSMXTETMP	7	2	54,10.5
28	AMPCBCMKMMHNANMSMXSMTETMP	11	2	54,3.5
29	AMPCBCMGMKMMHNASMXTETOBTMP	11	4	51,33,22,4
30	AMPCBCMGMKMMHNASMXSMTETOBTMP	12	3	54,36,26
31	AMPCBCMKMMHNANMSMXSMTETMP	11	3	54,36,6
32	TE	1	1	7.8
33	NATE	2	3	40,33,12.5
34	KMNANMSMTETMP	6	3	54,33,8.2
35	KMNANMTE	4	4	58,43,28,16.5
36	KMNANMSMXSMTETMP	7	4	54.43,23.14.5
37	KMMHNANMSMTE	6	1	6.8
38	KMMHNANMSMTE	6	1	6
39	NA	1	2	54,16.5
40	AMPCBNASMTE	5	1	38
41	AMPCBCMNMSMTE	6	1	23
42	NASMXSMTETOBTMP	6	1	22
43	CMMHNASMXSMTETMP	7	1	38
44	CMKMNANMSMXSMTETMP	8	2	45,33
45	AMPCBCMKMNANMSMXSMTETOBTMP	11	2	58,48
46	AMPCBMHSMTE	5	2	48,38
47	AMPCBMHSMTE	5	2	51,38

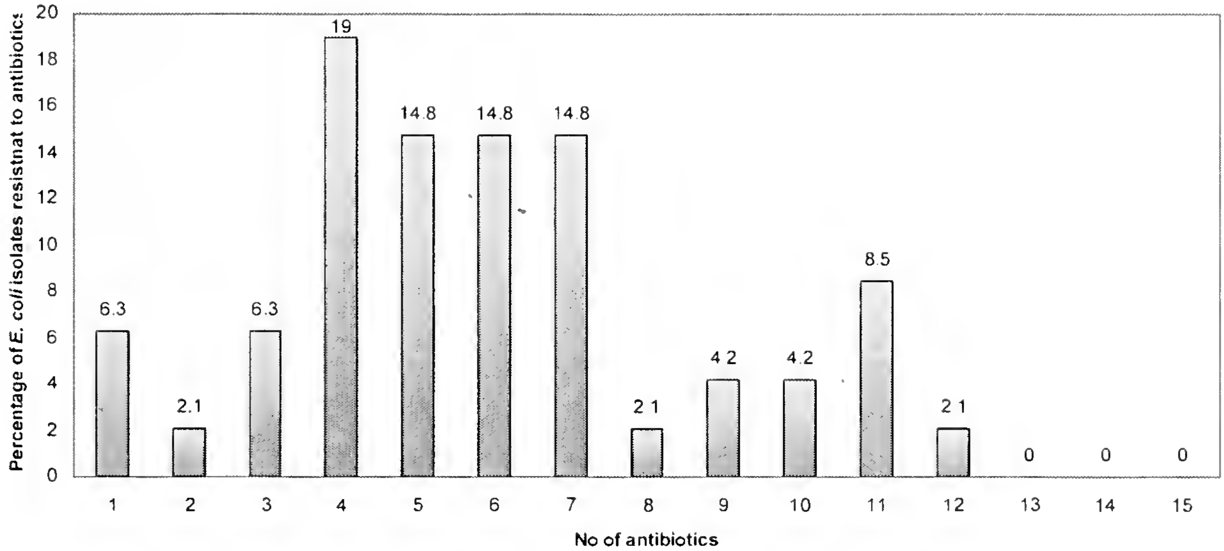


Figure 1. Percentage of *Escherichia coli* isolates resistant to multiple antibiotics.

Figure 2 also shows the antibiotic resistance patterns of the isolates. Most of the isolated strains shared resistance to four antibiotics, namely kanamycin, nalidixic acid, streptomycin, and tetracycline and therefore exhibited a common resistance pattern of KMNASMTE. The highest frequency was with tetracycline (97.9%) followed by NA (78.7%), SM (68.1%) and KM (59.6%). None of the strains were resistant to AK and CTX (Fig. 2).

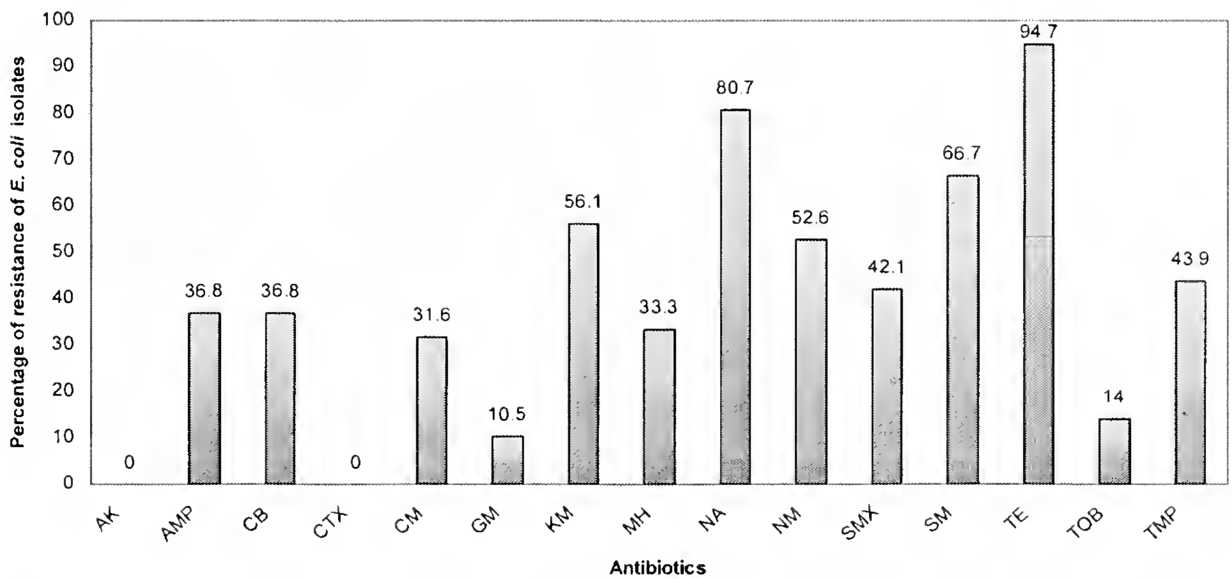


Figure 2. Percentage of *Escherichia coli* isolates resistant to each of the antibiotics tested.

A plasmid occurrence rate of 100% (47 out of 47) was observed. Plasmids analyses revealed that all resistant strains harbored 1-5 small size plasmids with molecular weight (m.w) in the range 2.9-66 kilo base (kb) (Table 1). Fifteen strains (31.9%) had one plasmid, 13 (27.7%) had two plasmids, 9 had three plasmids (19.1%), 8 strains had four plasmids (17%) and 2 (4.3%) harbored five plasmids. In general, strains resistant to one antibiotic contained one plasmid. However, strain 9 resistant to TE contained two plasmids (58 and

26 kb). Also, some of the isolates were resistant to seven antibiotics, strain 12, and strain 23 resistant to 9 antibiotics but contained 1 plasmid each, 3.3 kb and 43 kb respectively.

DISCUSSION

Reinthaler et al.., (2003) found that most *Escherichia coli* strains from sewage exhibited multiple resistance to antibiotics. Multiple resistance was reported to be more common than resistance to single antibiotics (Caudry and Stanisich, 1979). It was also reported that a high percentage of *Escherichia coli* (86.5%) isolated from avian feces were resistant to one or more antibiotics (Tabatabaei and **Nasirian**, 2003). In this study, all of the isolates were resistant to at least one antibiotic. Tetracycline resistance was also observed among *Escherichia coli* isolates and has been frequently reported in poultry products (Sackey et al., 2001). Tabatabaei and Nasirian, 2003 reported that 94% of *Escherichia coli* isolates from chickens were resistant to TE and 46% were resistant to KM. Their findings are similar to our results. Kariuki et al., (1999) reported that TE is one of the broad-spectrum antibiotics that are available in feed supplements, and its improper use led to the development of multiple antibiotic resistances. In the United States, resistance to TE increased from 9% in 1980 to 24% in 1990 (Winokur et al., 2000). Hofacre et al.., (2002) reported that 90% of *Escherichia coli* poultry isolates were resistant to TE. In Jamaica, 82.4% of *Escherichia coli* isolates were resistant to tetracycline (Miles *at al.,* 2006). Reinthaler et al., (2003) showed that resistance to TE can be transferred into the environment.

The percentage resistance to NA (78.8 %) in our study was higher than that reported in Malaysia (13%) (Radu et al., 2001) and the United States (37 %) (Johnson *et al.*., 2003). In the present study, *Escherichia coli* strains were found to be resistant to CM and GM, whereas in Sweden, Ronner *et al.*., (2004) found that chicken isolates were not resistant to CM and GM. Aja et al., (2002) reported that some of their isolates were resistant to AK, but our study showed that our strains were not resistant to AK.

The high rates of resistance found in this study can be explained by the wide spread use of antibiotics in Oman for prophylaxis and for treatment in poultry farms. The improper and unnecessary use of antimicrobial drugs in man also promotes development of resistant strains with R-plasmids. Linton *et al.*., (1977) reported that both pathogenic and non-pathogenic strains resistant to drugs may be transported from animals to humans via food. Such strains act as an important source for *in vivo* transmission of R-plasmids to drug sensitive strains in the animal intestine mainly through conjugation (Platt et al., 1986). A great similarity between the plasmids of *Enterobacteriaceae* isolated from animals and humans has been observed. Other workers reported that transmission of resistance plasmids of *Escherichia coli* from poultry to human intestines commonly occurs (Tabatabaei and Nasirian, 2003).

The plasmid DNA analysis of the strains in this study showed that the size of the plasmid DNA varied. Although some strains were resistant to only one antibiotic, they had more than one plasmid while others containing 1 or 2 plasmids were resistant to a large number of antibiotics. Al-Bahry (2000) reported similar findings. In his study, plasmid DNA analysis of the 28 *Salmonella* strains showed that the size of the plasmid DNA ranged from 3.1 kb to 32 kb. A study by Son et al., (1997) on isolates from fish revealed a similar size range of R plasmids (3 to 63.4 kb).

Nine strains (19%) were found to harbor 54 kb plasmids. Icgen et al., (2002) reported that a common 54 kb plasmid was harbored by 55% of local isolates of *Enterobacteriaceae* from Turkey. Al-Bahry (2000) reported that most strains associated with non-human sources were found to harbor larger plasmids while most human strains have relatively much smaller plasmids with sizes 10.8, 9, 4.7 and 6.2 kb pairs, yet they were resistant to a larger number of antibiotics. This suggests that not all antibiotic resistance genes are located in plasmids. Some of the genes conferring resistance may be located on bacterial chromosome. Aja et al., (2002) in their study of *Vibrio* strains isolated from cultured shrimps reported that some strains were resistant to four antibiotics, others were resistant to two antibiotics and all contained one plasmid of 21.2 kb pair. They suggested that resistance to antibiotics could be encoded in some strains in plasmids and in others in the chromosomes.

It is well established that antibiotic pressure supports resistant strains and eliminates sensitive strains. The greater the overuse of antibiotics, the more the elimination of the sensitive strains allowing resistant strains to dominate. It is also true that resistant strains are outcompeted by sensitive strains when antibiotic pressure is removed from the environment. Thus, steps must be taken to control the overuse of antibiotics in Oman as well as in other developing countries.

LITERATURE CITED

Aja, A. M., A. G. Gasca, A. A. Grobois, C. B. Mejia, A. Roque, and B. G. Gil. 2002. Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. *FEMS Microbiology Letters*. 213: 7-12.

Al-Bahry, S. N. 2000. Plasmid profiling of antibiotic resistant *Salmonella* species isolated in Muscat, Oman. *Pakistan Journal of Biological Sciences.* 3: 215-218.

Al-Ghamdi, M. S., F. Al-Ramadhan, and M. Hanif. 1999. Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in eastern province of Saudi Arabia. *Tropical Medicine and Internal Health.* 4: 278-283.

Bauer, A., W. Kirby, W. Sherris, and M. Turk. 1966. Antibiotic susceptibility testing by standard single disk method *American Journal of Clinical Pathology*. 45: 493-496.

Carraminana, J. J., C. Rota, I. Agustin, and A. Herrera. 2004. High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from a poultry slaughterhouse in Spain. *Veterinary Microbiology.* 104: 133-139.

Caudry, S. D., and V. A. Stanisich. 1979. Incidence of antibiotic-resistant *Escherichia coli* associated with frozen chicken carcasses and characterization of conjugative R plasmids derived from such strains. *Antimicrobial Agents and Chemotherapy.* 16: 701-709.

Chee-Sanford, J. C., R. S. Aminov, I. J. Krappac, N. Garrigues-Jeanjean, and R. I. Mackie. 2001. Occurrence and diversity of tetracycline resistance gene in lagoons and groundwater underlying two swine production facilities. *Applied Environmental Microbiology.* 67: 1494-1502.

Cordano, A. M., and R. Virgilio. 1996. Evolution of drug resistance in *Salmonella panama* isolates in Chile. *Antimicrobial Agents and Chemotherapy.* 40: 336-341.

Elwell, L., and S. Falkows. 1980. The characterization of plasmids that carry antibiotic

resistance genes, p. 433-453. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. Williams and Wilkins. Baltimore.

Hofacre, C. L., M. Ginevan, R. Carnevale, C. Thrnsberry, E. Gonder, G. Tillotson, M. Pasternack, E. Rubinstein, D. Newell, and T. Wassenaar. 2002. The health and management of poultry production. *Int. The Journal of Infections Diseases.* 6:353-357.

Icgen, B., G. C. Guerakan, and G. Oezcengiz. 2002. Characterisation of local isolates of *Enterobacteriaceae* from Turkey. *Microbiological Research.* 157: 233-238.

Johnson, J. R., A. C. Murry, A. Gajewski, M. Sullivan, P. Snippes, M. A. Kuskwvski, and K. E. Smith. 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrobial Agents and Chemotherapy.* 47: 2161-2168.

Kariuki, S., C. Gilks, J. Kimari, A. Obanda, J. Muyodi, P. Waiyaki, and C. A. Hart. 1999. Genotype analysis of *Escherichia coli* strains isolated from children and chickens living in close contact. *Applied Environmental Microbiology.* 65: 472-476.

Linton, A., K. Howe, P. Bennet, M. Richmond, and E. Whiteside. 1977. The colonization of the human gut by antibiotic resistance *E. coli* from chickens. *Journal of Applied Bacteriology.* 43: 465-469.

Miles, T. D., W. McLaughlin and P. D. Brown. 2006. Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *BMC Veterinary Research.* 2: 7

Mirza, S., S. Kariuki, K. Z. Mamun, N. J. Beeching, and C. S. Hart. 2000. Analysis of plasmid and chromosomal DNA of multidrug-resistant *Salmonella enterica* serovar Tuphi from Asia. *Journal of Clinical Microbiology.* 38: 1449-1452.

Osterblad M., A. Hakanen, R. Manninen, T. Leistevuo, R. Peltonen, O. Meurman, P. Huovinen, and P. Kotilainen. 2000. A between-species comparison of antimicrobial resistance in Enterobacteria in fecal flora. *Antimicrobial Agents and Chemotherapy.* 44: 1474-1484.

Platt, D., D. Brown, and D. Munro. 1986. The distribution of plasmids among a representative collection of Scottish strains of *Salmonella. Journal of Hygiene.* 97:199-204.

Radu, S., O. W. Ling, G. Rusul, M. I. Abdul-Karim, and M. Nishibuchi. 2001. Detection of *Escherichia coli* O157:H7 by multiplex PCR and their characterization by plasmid profiling, antimicrobial resistance, RAPD and PFGE analyses. 2001. *Journal of Microbiological Methods.* 46: 131-139.

Reinthaler, F. F., J. Posch, G. Feierl, G. Wust, D. Haas, G. Ruckenbauer, F. Mascher, and E. Marth. 2003. Antibiotic resistance of *E. coli* in sewage and sludge. *Water Research.* 37: 1685-1690.

Rice, E. W., J. W. Messer, C. H. Johnson, and D. J. Reasoner. 1995. Occurrence of high-level aminoglycoside resistance in environmental isolates of *Enterococci. Applied and Environmental Microbiology.* 61: 374-376.

Roche Applied Science. 2003. High pure plasmid isolation kit. Roche. Germany.

Ronner, A. C., E. O. Engvall, L. Andersson, and B. Kaijser. 2004. Species identification by genotyping and determination of antibiotic resistance in *Campylobacer jejuni* and *Campylobacter coli* from humans and chickens in Sweden. *International Journal of Food Microbiology.* 96: 173-179.

Sambrook, J., E. F. Fritch and T. Maniatis. 1989. Molecular Cloning. A laboratory manual. Second edition. Cold Spring Harbor laboratory Press.

Sackey, B. A., P. Mensah, E. collision, and E. Sakyi-Dauwson. 2001. *Campylobacter, Salmonella, Shiegella* and *Escherichia coli* in live and dressed poultry from metropolitan Accra. *International Journal of Food Microbiology.* 71: 21-28.

Saenz, Y., M. Zarazaga, M. Lantero, M. J. Gastanares, F. Baquero, and C. Torres. 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. *Applied and Environmental Microbiology.* 44: 267-271.

Saxena, S., M. Mago, N. Kumari, and L. Rao. 1984. R-plasmid of some isolated *Salmonella* serotypes. *Indian Journal of Medical Research.* 79: 307-311.

Smalla, K., H. Heuer, A. Gotz, D. Niemyer, E. Krogerrecklenfort, and E. Tietze. 2000. Exogenous isolation of antibiotic plasmids from piggery manure slurries reveals a high prevalence and diversity of IncQ- like plasmids. *Applied and Environmental Microbiology.* 66: 4854-4862.

Son, R., G. Rusul, A. M. Sahilah, A. Zainuri, A. R. Raha, and I. Salmah. 1997. Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Telapia (*Telapia mossambica*). *Letters in Applied Microbiology.* 24: 479.

Sonnenwirth, A. C., and L. Jarett. 1980. Gradwohl's clinical laboratory methods and diagnosis. Vol. 2. 8th Edition. C. V. Mosby Company. St. Louis.

Tabatabaei, R. R., and A. Nasirian. 2003. Isolation, Identification and Antimicrobial Resistance Patterns of *E. coli* Isolated from Chicken Flocks. *Iranian Journal of Pharmacology and Therapeutics.* 22: 39-42.

Winokur, P. L., A. Brueggernann, D. L. Desalvo, L. Hoffmann, M. D. Apley, E. K. Uhlenhopp, M. A. Pfaller, and G. V. Doern. 2000. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC b-Lactamase. *Antimicrobial Agents and Chemotherapy.* 44: 2777–2783.

MOBILIZATION OF MERCURY FROM CONTAMINATED SOIL IN TO AN AQUATIC FOOD CHAIN

Candice R[1]. Kohute, Alfred C. Nichols[2], David A. Steffy[2], and Mark E. Meade[1]
Department of Biology[1] and Department of Physical and Earth Sciences[2]
700 Pelham Road North, JSU Box 7990
Jacksonville State University
Jacksonville, Alabama 36265

Correspondence: Kohute, Candice (jsu6200d@jsu.edu)

ABSTRACT

Fish were collected from a site with mercury (Hg) contaminated soil to determine if Hg was moving from the soil into the aquatic food chain. Fish were collected from a site in Oxford, AL, where Snow Creek, a third-order perennial creek, passes through Hg contaminated soil. Fish were also collected upstream where the soil Hg concentration is at background. Fill deposits used as landscape material in the construction of Oxford Park may have been contaminated with Hg from industrial sources. This land now serves as a non-point source of Hg contaminated soil. Soil samples from the park have a mean Hg content of 1.195 mg/Kg, compared to a background value for the area of 0.039 mg/Kg. Sediment samples taken from Snow Creek as it passes through the park have a mean Hg content of 0.319 mg/Kg. Fish were collected from Snow Creek as it passes through the park and from the upstream site. Fish were analyzed for total Hg using cold vapor atomic absorption. Significant differences were seen in the Hg levels between fish species collected from the park and those collected from the upstream site. The data indicates that Hg from the park is entering the aquatic food chain of Snow Creek.

INTRODUCTION

Mercury (Hg) has been a global environmental pollutant for several decades and is a worldwide health concern. In the Clean Air Act, the United States Environmental Protection Agency (USEPA) identified Hg as the pollutant posing the greatest threat to human health (Gray et al., 2004). Mercury is continuing to contaminate fish in rivers, streams, and lakes across the United States. The metal can enter the aquatic environment from either natural or anthropogenic point sources. Sometimes no direct source of the Hg can be determined. The major Hg contamination source for remote rivers, streams, and lakes is thought to be atmospheric transport and deposition of atmospheric Hg (Lange et al., 1993). Advisories for fish consumption due to elevated Hg contamination are a

common phenomenon in many regions of the United States (Balogh et al., 1998a). The contamination of Hg in fish from affected streams remains decades after the use of Hg has ceased, often after remediation efforts were assumed successful (Southworth et al., 2000). Bioaccumulation of Hg in fish can be hazardous to both human and wildlife consumers (Balogh et al., 1998b). Commercial and sport fish, which frequently contain the highest concentrations of Hg, are often used to monitor the bioaccumulation of Hg in the aquatic ecosystems (Peterson et al., 1996).

In the United States, methylmercury is one of the most widespread contaminants of aquatic ecosystems. Methylmercury is a potent neurotoxin to piscivorous wildlife and also poses a serious threat to humans (USGS, 2001). While Hg can be retained in soil, it also can leach into surface and subsurface waters (Paller et al., 2004). Although Hg usually enters the aquatic environment in an inorganic form (Nichols et al., 2002), it is converted to methylmercury by microorganisms before entering the aquatic food chain (Ramlal et al., 1986). Methylmercury is transferred from the sediment of streams, rivers, and lakes into the water by microorganisms and eventually into the biota. Methylmercury is a water soluble compound and is readily absorbed by organisms (Gray et al., 2004).

Any form of Hg can be converted to methylmercury by natural means and then bioaccumulate and biomagnify as it progresses through aquatic food webs. Methylmercury has a strong affinity for sulfur-containing organic compounds and is absorbed by fish from ingestion of dietary sources. Over 90% of Hg found in fish is in the form of methylmercury (Spry and Wiener, 1991). Mercury can bioaccumulate in fish populations as benthic organisms recycle Hg contaminated sediments (Nichols et al., 2002).

Snow Creek flows through highly urbanized areas of Anniston and Oxford, Alabama. The stream is third-order perennial and is relatively calm with alternating pools and riffles. This area includes a shopping mall parking lot and a city park in Oxford, Alabama (Fig. 1).

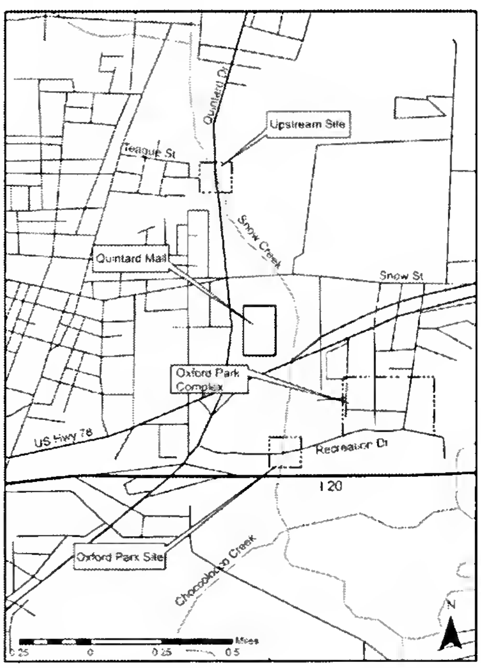


Figure 1. Location of fish collection sites along Snow Creek at the upstream and Oxford Park locations in Anniston and Oxford, Alabama.

The city park contains a large lake, baseball fields, playground areas, and tennis courts and is on the floodplain of Snow Creek. Local newspapers have reported that sand from local foundries was used for landscape materials during construction of the city park and the shopping mall parking lot. This area currently serves as a non-point source of Hg contaminated soil. Approximately one mile downstream from the park, Snow Creek empties into the Choccolocco watershed. Choccolocco Creek and the surrounding floodplain have gradually been contaminated with Hg through erosion of contaminated sediment from Oxford City Park (Nichols et al., 2005).

This study was conducted to determine if Hg from contaminated land fill was entering the aquatic food chain in Snow Creek. We previously analyzed 54 bias surface soil and sediment samples from 18 sites in the Snow Creek Watershed (Steffy and Nichols, 2005). Mercury-contaminated soil was found downstream from Quintard Mall and Oxford City Park in both the floodplain and in channel sediment deposits of the creek. Soil from the floodplain in the park had mercury levels at 1.195 ± 0.713 mg/Kg (mean $\pm$ SD). Channel deposits at the park and downstream have measured mercury levels at 0.319 ± 0.395 mg/Kg, whereas upstream channel sediment have levels at 0.070 ± 0.029 mg/Kg. Background soil samples taken outside of the floodplain have measured mercury levels at 0.039 ± 0.018 mg/Kg.

MATERIALS AND METHODS

Fish were collected from Snow Creek in Oxford Park and from an upstream site. The fish were collected between early spring and late fall of 2005 using backpack electrofishing (Model 12 POW Electrofisher). Fish were immediately euthanized by placing on ice. In the lab, fish were sorted by species using dichotomous keys, and wet weight was determined using an electronic balance recorded to the nearest 0.001 g. Once the fish were weighed and measured, they were stored in zip-seal bags at –17 °C until processing.

Whole fish sampling was conducted for each species. Stonerollers, a minnow size fish, were sampled in groups to obtain adequate tissue for testing. These small fish were divided into three groups by total length (cm). In each sample an average of 25 fish were placed in the 0.0 to 5.5 cm group, an average of 25 fish in the 5.5 to7.5 cm group, and an average of 20 fish in the 7.5 to 12.5 cm group. The other fishes, all of the family centrarchidae (common name "sunfishes") were processed individually. The fish were thawed and homogenized using 20 mL aliquot of ultrapure water (17 megaohm resistance) and a Waring Laboratory blender. Each sample was homogenized for 20-40 s on low speed. Homogenized samples were frozen at -70 °C for a minimum of 24 h. Tissue was then freeze-dried (VirTis Freezemobile 12) for 3-4 d. Triplicate freeze-dried samples from the same homogenate (approximately 2.0 g) were weighed using an electronic balance and placed in acid washed 150 mL beakers. The exact weight of each sample of dry tissue was recorded to the nearest 0.001 g using an electronic balance. Dry tissue samples were digested in a mixture of 15 mL of double-distilled nitric acid and 2 mL of 30% hydrogen peroxide at 45 °C on a hotplate under a fume hood. A reagent control treated in a similar fashion was included with each batch of tissue. The residue from each sample was dissolved in 7% nitric acid solution and filtered (Fisherbrand Q8 filter paper) into

an acid-washed 50 mL volumetric flask and diluted to 50 mL with 7% nitric acid. Fish tissue was further digested in sulfuric acid, nitric acid, potassium persulfate, and potassium permanganate solution by heating the mixture to 95 °C for 2 h. This treatment ensured that all forms of Hg were converted to the mercuric ion. Unreacted potassium permanganate was reduced by adding 1 mL of a 12% hydroxylamine solution to each sample.

The samples were analyzed for total Hg using USEPA Method 245.1, Manual Cold Vapor Technique (USEPA, 1983). Hg analysis was conducted using a CETAC Quick Trace Mercury Analyzer M-1600 cold vapor atomic absorption Hg analyzer with an ASX-400 AutoSampler. During flow injection, a 7% stannous chloride solution reduced all mercuric ions to elemental Hg. All specimens were run in batches that included blanks (reagent and instrument), a five point standard calibration curve (standards of 0.0, 0.5, 1.0, 2.5, 5.0, and 10.0 µg/L with a linear correlation of 0.999 or better), and spiked specimens. Matrix spikes gave 85-90% recovery. Specimen split between two batches had a variation of less than 5%.

InStat, version 3.0 for Windows (Graphpad Software, Inc., San Diego, CA) was used for data analyses which included Kolmogorov-Smirnot test for normality, the unpaired t-test, the Mann-Whitney test, one-way analysis of variance (ANOVA), Fisher's protected least significant difference test, the Krushkal-Wallis test for nonparametric ANOVA, and Dunn's multiple comparisons test.

RESULTS

Among the fish species collected from both sampling locations were longear sunfish (*Lepomis megalotis*), bluegill sunfish (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), redbreast sunfish (*Lepomis auritus*), and largescale stonerollers (*Campostoma oligolepis*). Mercury was detected in the tissue of all fish tested (Table 1). Only three species of fish were collected in numbers sufficient to perform statistical analysis between the park and the upstream sites. These were stonerollers, longear sunfish, and bluegill sunfish. Green sunfish and redbreast sunfish were only collected at the upstream location. The green sunfish had a mean Hg tissue concentration of 0.028 ± 0.006 µg/g (mean ± SD, dry weight). The redbreast sunfish had a mean Hg tissue concentration of 0.037 ± 0.011 µg/g (Fig. 2).

Table 1. Hg concentrations in fish collected from Oxford Park and upstream sites along Snow Creek in Anniston and Oxford, Alabama

Species	Location	Hg Concentration (µg/g, dry weight)
Stonerollers	Oxford Park	0.047 ± 0.009
	Upstream	0.031 ± 0.005
Longear sunfish	Oxford Park	0.121 ± 0.057
	Upstream	0.067 ± 0.015
Bluegill sunfish	Oxford Park	0.072 ± 0.054
	Upstream	0.032 ± 0.018
Redbreast sunfish	Upstream	0.037 ± 0.011
Green sunfish	Upstream	0.028 ± 0.006

Significant differences were seen in the Hg levels between fish collected from the park and fish collected from the upstream site. The largescale stonerollers, *Campostoma oligolepis*, had the lowest Hg concentrations at both sampling locations. Stonerollers collected upstream from the park had a mean Hg concentration of 0.031 ± 0.005 μg/g (Fig. 2), whereas those collected from the park had a mean Hg tissue concentration of 0.047 ± 0.009 μg/g (Fig. 3). An unpaired t-test on these groups resulted in a two-tailed P value of < 0.001.

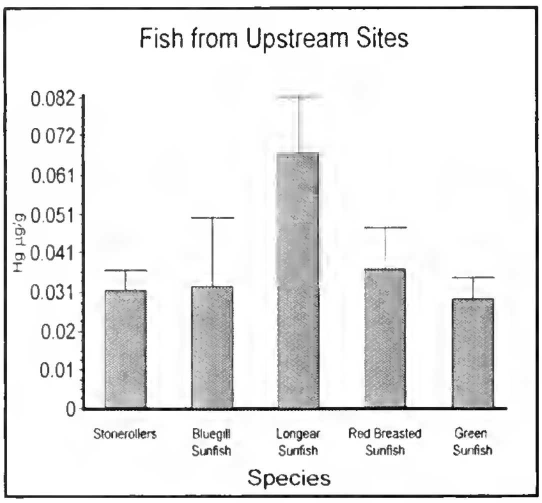


Figure 2. Hg concentrations among fish species collected at upstream along Snow Creek in Anniston and Oxford, Alabama.

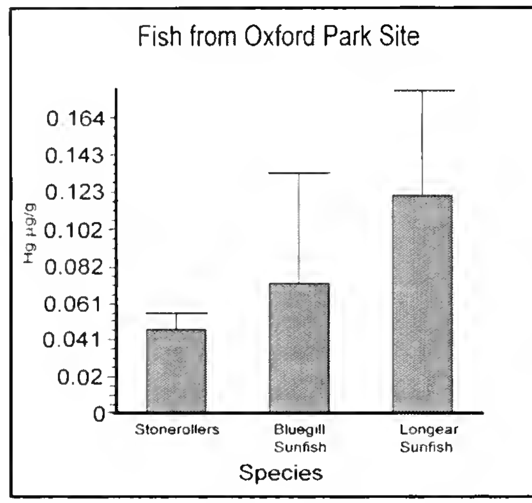


Figure 3. Hg concentrations among fish species collected at Oxford Park along Snow Creek in Anniston and Oxford, Alabama.

Bluegill sunfish, *Lepomis macrochirus*, and longear sunfish, *Lepomis megalotis*, had Hg tissue concentrations higher than those seen in stonerollers. Bluegill sunfish collected upstream had a mean Hg concentration of 0.032 ± 0.018 μg/g (Fig. 2). The bluegill sunfish collected from the park had a mean Hg concentration of 0.072 ± 0.054 μg/g (Fig. 3). An unpaired t-test conducted on these two groups produced a two-tailed P value of 0.012, significant at $P < 0.05$.

Longear sunfish accumulated the highest amount of Hg among all fish collected in the study. The longear sunfish had a mean Hg tissue concentration of 0.067 ± 0.015 μg/g at the upstream site (Fig. 2). The mean Hg tissue concentration of longear sunfish from the upstream sampling site was twice that seen in stonerollers and bluegill sunfish. Longear sunfish collected from the park had a mean Hg tissue concentration of 0.121 ± 0.057 μg/g (Fig. 3), which was three times the Hg tissue concentration found in stonerollers. An unpaired t-test conducted on longear sunfish from the park and upstream resulted in a two-tailed P value of 0.014, significant at $P < 0.05$.

When Hg concentrations in bluegill and longear sunfish collected from the park and from upstream were compared using ANOVA, the ANOVA F value was 12.016 with a P value of 0.0012. Fisher's protected least significant difference test showed significant difference at $P < 0.05$ between the park and upstream sunfish. The mean Hg concentration for park sunfish was 0.091 ± .060 μg/g, and for upstream bluegill and longear sunfish combined, the mean Hg level was 0.045 ± 0.01.

Mercury concentrations in four different fish species collected at the upstream site were compared using the Kruskal-Wallis test (nonparametric ANOVA). This generated a P value of < 0.0001 and a Kruskal-Wallis statistic KW = 20.260. Dunn's multiple comparisons test showed significant differences between bluegill and longear ($P < 0.001$), between longear and redbreast ($P < 0.05$), and between longear and green sunfish ($P < 0.01$). In each comparison, the longear sunfish had significantly higher Hg levels.

The Mann-Whitney test was used to compare Hg levels between the two different sunfish species collected from Oxford Park. Bluegills collected in the park had a mean Hg level of 0.072 ± 0.054 µg/g, and longear had a mean of 0.122 ± 0.057. The Mann-Whitney U-statistic for this comparison was 22.500, which gave a two-tailed P value of 0.008. The longear sunfish accumulated more Hg under both high and low environmental exposures. At the upstream site, there was no significant difference in Hg levels among bluegills, redbreast, and green sunfish.

DISCUSSION

Mercury was detected in all fish collected in this study. The fish collected were representative of area streams. All the fish collected appeared healthy. In a previous study conducted in northwest Alabama involving catfish collected from rivers, lakes, and ponds detectable levels of Hg were found in all fish. The Hg levels of the catfish collected from the two impoundments of the Tennessee River ranged from 0.05 µg/g to 3.28 µg/g (dry weight) in the liver tissue and 0.01 µg/g to 0.21 µg/g in the muscle tissue. The Hg levels of the catfish collected from the neighboring ponds ranged from 0.03 µg/g to 4.79 µg/g in the liver tissue and 0.01 µg/g to 0.54 µg/g in the muscle tissue. The Hg levels in the fish were attributed to atmospheric depositions from coal-burning power plants (Nichols et al., 2002). Mercury emissions from electric utilities are the largest anthropogenic source of Hg in the atmosphere. Approximately 51 tons of Hg are emitted nationwide each year from coal-burning power plants (USEPA, 1998). Atmosphere deposition from area coal-burning power plants could account for the Hg detected in fish collected at the upstream site.

A significant difference was seen in the Hg levels between fish collected upstream and those collected in Oxford Park for each of the three species examined. Stonerollers had the lowest Hg concentrations at both locations. This was expected, as they are a primary consumer in the aquatic food chain (Boschung and Mayden, 2004; Mettee et al., 1996). Bluegill and longear sunfish are opportunistic carnivores and, as such, are higher up the aquatic food chain than stonerollers. Both species had Hg tissue concentrations higher than those seen in stonerollers. Longear sunfish accumulated more Hg than the other species at both the low concentration and high concentration sites.

Snow Creek is a perennial stream. During the time specimens were collected, pools in the creek ranged from 2 to 3 feet in depth. As the sunfish increase in size, they tend to migrate to deeper water downstream, in this case to an impoundment lake (Logan-Martin) on the Coosa River. Sunfish are popular for local sports fishing (especially for children as the fish are easily caught from the bank) and eating. Also, as sunfish grow and migrate into deeper waters of the Coosa River, they become prey for larger fish. This can result in biomagnification as Hg moves up the food chain. While the Hg levels found in this study

are below those considered hazardous for human consumption (1.0 μg/g, wet weight), it is disturbing that detectable levels of the metal are present at all. For each species tested, Hg levels were significantly higher in fish obtained from Oxford Park than those found in fish sampled from upstream. We have previously presented data showing that Hg levels above background can be found in creek sediments as far as 14 miles downstream from Oxford Park (Steffy and Nichols, 2005). We conclude that Hg contained in contaminated land fill has not been immobilized by interactions with the soil. On the contrary, our data indicates that Hg from Oxford Park is slowly leaching into Snow Creek where it is entering the aquatic food chain as Snow Creek drains into Lake Logan Martin and the Coosa River system.

ACKNOWLEDGEMENTS

The authors would like to thank Jacksonville State University for providing funding for this research.

LITERATURE CITED

Balogh, S. J., M. L. Meyer, and D. K. Johnson. 1998a. Transport of mercury in three contrasting river basins. *Environmental Science and Technology*. 32: 456-462.

Balogh, S., M. Meyer, and K. Johnson. 1998b. Diffuse and point source mercury inputs to the Mississippi, Minnesota, and St. Croix Rivers. *The Science of the Total Environment*. 213: 109-113.

Boschung, H. T., and R. L. Mayden. 2004. *Fishes of Alabama*. Smithsonian Books, Washington D. C., USA.

Gray, J. E., M. E. Hines, P. L. Higueras, I. Adatto, and B. K. Lasorsa. 2004. Mercury speciation and microbial transformations in mine wastes, stream sediments, and surface waters at the Almaden mining district, Spain. *Environmental Science and Technology*. 38: 4285-4292.

Lange, T. R., H. E. Royals, and L. L. Connor. 1993. Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. *Transactions of the American Fisheries Society*. 122: 74-84.

Mettee, M. F., P. E. O'Neil, and J. M. Pierson. 1996. *Fishes of Alabama and the Mobile Basin*. Oxmoor House, Birmingham, Alabama, USA.

Nichols, A. C., T. P. Murray, and T. D. Richardson. 2002. Mercury accumulation in catfish, *Ictalurus furcatus* and *I. punctatus*, from the Southeastern Tennessee River Valley. *Southeastern Naturalist*. 1: 159-168.

Nichols, A. C., D. A. Steffy, and S. Al-Hamdani. 2005. Mercury distribution in sediments and uptake by plants along Snow and Choccolocco Creeks in Northeast Alabama. *The Toxicologist*. 84: 324.

Paller, M. H., C. H. Jagoe, H. Bennett, H. A. Brant, and J. A. Bowers. 2004. Influence of methylmercury from tributary streams on mercury levels in Savannah River Asiatic clams. *Science of the Total Environment.* 325: 209-219.

Peterson, M. J., G. R. Southworth, and W. D. Crumby. 1996. Monitoring mercury in fish in a stream system receiving multiple industrial inputs. *Environmental Monitoring and Assessment.* 40: 91-105.

Ramlal, P. S., J. W. M. Rudd, and R. E. Hecky. 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. *Applied and Environmental Microbiology.* 51: 110-114.

Southworth, G. R., R. R. Turner, M. J. Peterson, M. A. Bogle, and M. G. Ryon. 2000. Response of mercury contamination in fish to decreased aqueous concentrations and loading of inorganic mercury in a small stream. *Environmental Monitoring and Assessment.* 63: 481-494.

Spry, D. J., and J. G. Wiener. 1991. Metal bioavailability and toxicity to fish in low alkalinity lakes: a critical review. *Environmental Pollution.* 71: 243-304.

Steffy, D.A., and A. C. Nichols. 2005. Mercury occurrence in the Middle Choccolocco Creek Watershed, Calhoun County, Alabama, presented at the Southeast Regional Meeting for the Society of Environmental Toxicologists and Chemists, Dauphin Island, AL.

USEPA. 1983. EPA methods for chemical analysis of water and wastes. Mercury, Method 245.1 Manual Cold Vapor Technique. U. S. Environmental Protection Agency.

USEPA. 1998. Mercury emissions and electric utilities. U. S. Environmental Protection Agency.

USGS. 2001. A national pilot study of mercury contamination of aquatic ecosystems along multiple gradients: bioaccumulation in fish. U. S. Geological Survey.

Journal of Alabama Academy of Sciences Vol. 77, No. 3-4, July/October 2006

APPLICATIONS OF LASER INDUCED BREAKDOWN SPECTROSCOPY

Akshaya Kumar and Prakash C. Sharma
Department of Physics, College of Engineering, Architecture and Physical Sciences
Tuskegee University, Tuskegee, AL 36088

Correspondence: Sharma, Prakash (PCSHARMA@tuskegee.edu)

ABSTRACT

Applications of laser induced breakdown spectroscopy (LIBS) in solid and liquid phases have been presented. Use of single pulse and double pulse LIBS to enhance the detection limit of LIBS has been desctribed. A correlation between plasma temperature and the LIBS signal in liquid phase has been presented. Potentials of LIBS in biomedia for example hair and nail have been presented. A difference in LIBS spectrum for normal and malignant tissue has been shown.

INTRODUCTION

LIBS is a technique that is able to analyze any matter in solid (Marquardt B.J et al., 1996), liquid (Kumar et al., 2003) or gaseous (Martin M. et al., 2000) form using an intense pulse of laser beam (Yueh, F.Y et al., 2000). In LIBS, a high power pulsed laser (typically in the range 10-100 mJ) is used to create a high temperature plasma spark (typically >10,000 Kelvin) which in turn vaporizes a small amount of the target sample material (typically micro-gram to pico-gram). This technique is also known as LIPS or Laser Induced Plasma Spectroscopy. The target sample material emits light that is characteristic of the elemental composition (duration less than ~10 ms). The excited plasma subsequently cools down, and the light emitted from the laser-created plasma is helpful in diagnosing the samples. The spectrum is then captured using a spectrometer in the 200 to 900 nm wavelength range. The emission from the plasma is fed to the spectrometer and the CCD detector to record the atomic signature of different elements present in the material.

Understanding the plasma dynamics is becoming more and more important because of the large number of technological applications in different areas of science and technology. Some of the research fields that include the plasma physics are material science, fluid dynamics, chemical physics, biology, space propulsions, and astro-physics (Margetic Vet al., 2003). The basic goal in different areas of research is to discover the different plasma parameters and harness plasma for useful purposes. Using lasers is one of the easiest ways to create plasma from any material.

Currently, laser produced plasma of solid and liquid material is of utmost interest, especially in the fields of laser diagnostics, laser fusion, thin film growth and chemical analysis. Laser Induced Breakdown Spectroscopy (LIBS) is an analytical technique in which laser light is used to create the plasma and the emission of the plasma is used to

recognize the composition of the material. (Rusak, D.A., 1997; Singh et al., 1997). The shot-to-shot variation of this technique can be high, but spectral samples can easily be captured at rates of hundreds of hertz, producing good statistical results. Some of the major advantages to this technique lie in its simplicity. Little or no sample preparation is required, reducing the possibility of contamination. It is minimally invasive since a very small amount of sample can give good results. Since data are easily interpretable, skilled analysts are not required. The instruments themselves can be made rugged and portable. This technique is quite similar to discharge/arc emission spectroscopy in which elements are excited to a higher energy state by the electric discharge. Using a laser beam, it is possible to create the discharge like situation. A highly focused laser beam is directed onto the surface of the sample. The intense electric field of the laser light creates the breakdown and the plasma in the sample. The basic principle of laser induced ablation is shown in Figure 1.

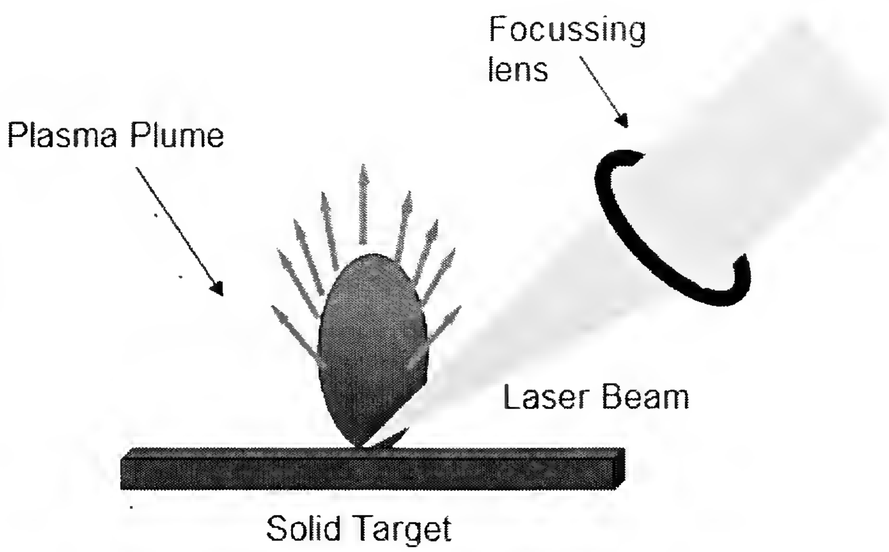


Figure 1. Laser ablation and plume of the solid material.

MATERIALS AND METHODS

It is possible to generate the LIBS signal using a high energy pulsed Nd:YAG laser or an excimer laser. Typical experimental arrangement for recording the LIBS spectra of liquid samples is shown in Figure 2. Laser light from a Q-switched frequency doubled Nd: YAG laser (Continuum Surelite III) that delivers energy up to 400 mJ in 8 ns time duration at 532 nm was focused on the center of the liquid mist stream of the nebulizer (Meinhard TR-30-C6) using a 20 cm focusing lens. The laser was operated at 10 Hz frequency, and its beam diameter was about 9 mm. In order to measure laser power, a OPHIR-OPTICS model no. 30A-P-CAL power meter can be used. In the given arrangement for liquid samples, a Meinhard nebulizer was originally designed to work in an optimized condition of 30 psi gas pressure, a gas flow rate of about 1000 ml/min, and a liquid flow rate of 5.7 ml/min for ICP measurements. However, in the case of LIBS, we observed that a liquid flow rate of

3.5 ml/min and a gas flow rate of 300 ml/min was the optimum for a good signal. In this experimental arrangement, a capillary of an internal diameter of ~ 0.22 - 0.32 mm carrying the liquid has been used. The same nebulizer can be used to create a fine jet if one stops purging the gas and increases the liquid flow rate. In the present experimental condition, we also recorded the LIBS signal for magnesium in the jet mode. The flow rate of liquid in the jet mode was 17.5 ml/min. The mist produced by the nebulizer is an aerosol that exits the tip at a very high speed, drops vertically approximately 30 mm (depending on gas and liquid flow rate), and finally disperses. The laser was focused on the center of the mist around three mm below the tip of the nebulizer to achieve a stable and reproducible signal. The closed loop system has been used for circulating the solution. Emission collected in the backward direction was coupled to an optical fiber bundle via two UV-grade quartz lenses of focal length 50 cm and 10 cm. The light entrance slit width of the spectrometer was 100 μm. The fiber bundle that collects the emission from the plasma plume was a collection of 80 single fibers of 0.1 mm core diameter. The other end of the fiber was connected to the entrance slit of a remote spectrograph (Model HR 460, Instrument SA, Inc., Edison, NJ). Finally, emitted light was dispersed by a 2400-l/mm diffraction grating of dimension 75 mm × 75 mm and converted into an electrical signal by an Intensified CCD camera (Princeton Instruments Corporation, Princeton, NJ) connected to the exit end of the spectrograph. The typical gate delay used for recording the calibration curve was chosen from the experimental condition that gave the best signal-to-noise and signal-to-background ratio. Noise here is defined as the standard deviation of the background signal. The typical spectra were recorded between 1 to 10 μs at a constant gate width of 10 μs. The signal was processed and stored in a computer. One hundred laser shots were accumulated to obtain one spectrum, and thirty spectra were recorded under the same experimental conditions to increase the sensitivity and reduce the standard deviation.

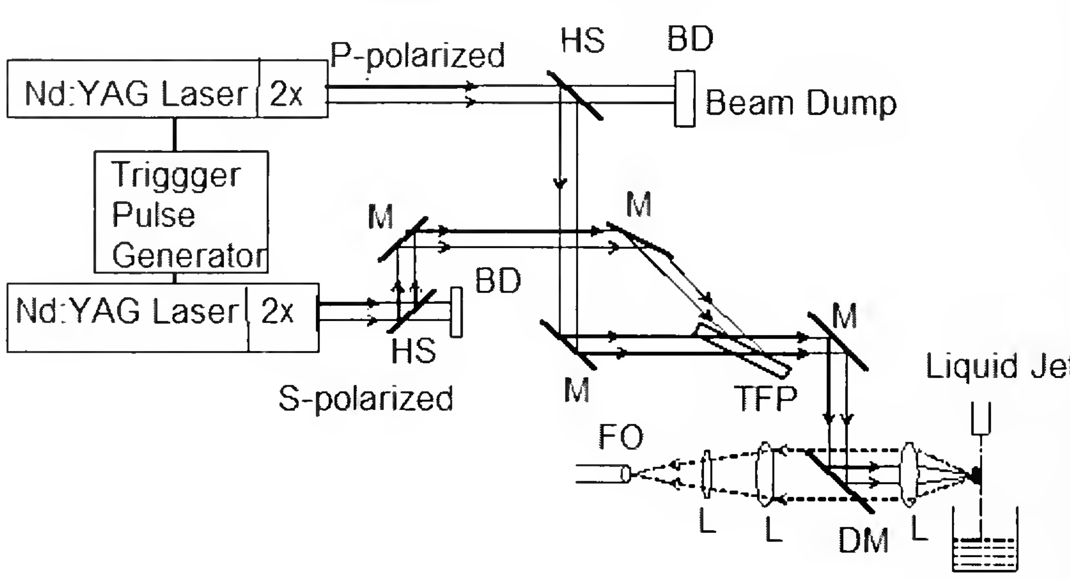


Figure 2. Experimental Setup to record the Applications of laser induced breakdown spectroscopy spectrum of liquid solution. [TFP: Thin Film Polarizer, DM: Dichroic mirror, HS: Harmonic Separator, M: Mirror, L: Lens, BD: Beam Dump and 2X: Second Harmonic].

Determination of the Elements Present in Solid and Liquid Phases:

Before starting to monitor the concentration of trace elements in the real samples, the LIBS system must be calibrated. The calibration curve for the elements can be obtained by plotting the signal intensity versus the concentration of the elements. The slope of the calibration curve along with the standard deviation in the signal decides the Limit of Detection (LOD) of the apparatus. LOD is defined as the minimum concentration of the element when the signal is three times more intense than the background noise fluctuation. The fluctuation in the background signal and the original characteristic signal lying on top of the background fluctuates in almost the same proportion. Mathematically, the LOD is defined as LOD = $3\sigma/R$, where σ is the standard deviation in the background and R is the slope of the signal intensity versus concentration curve. The calibration curve of Cr signal is shown in Figure 3.

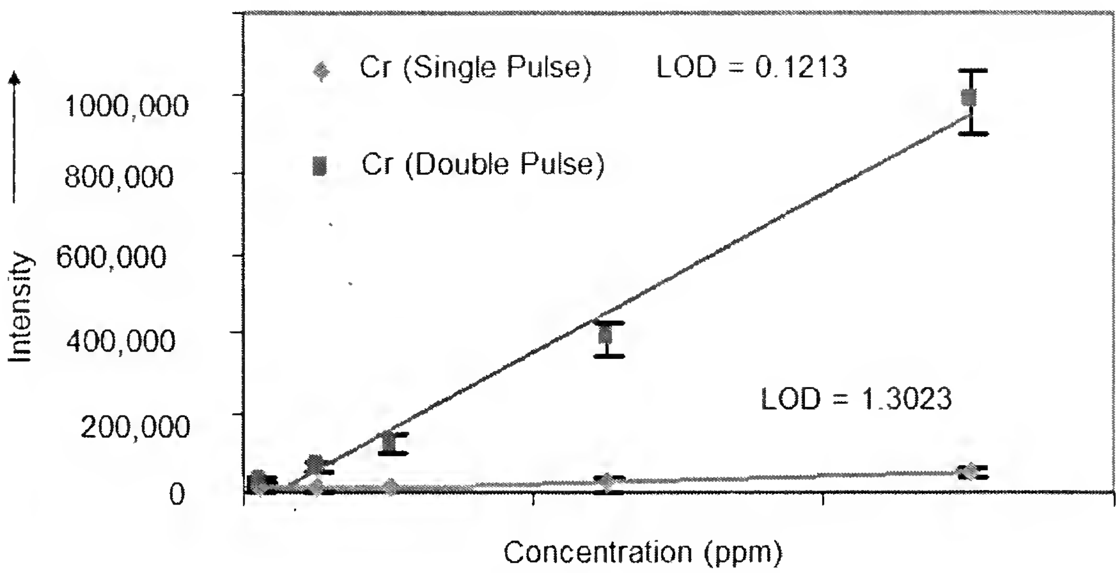


Figure 3. Calibration curve for chromium using single and double laser pulse of laser beam.

RESULTS AND DISCUSSIONS

Application of Applications of Laser Induced Breakdown Spectroscopy in Liquid Medium:

Applications of laser induced breakdown spectroscopy of liquids presents more problems than do solids because of higher breakdown threshold, splashing of liquid and turbulences causing loss of laser energy. Different techniques such as LIBS on liquid jet both thick and thin modes, application of magnetic field around the jet (Rai et al., 2003) and use of two sequential laser pulses have been adopted to improve the technique for liquid application.

Using double pulse technique, a significant enhancement in the LIBS signal (approximately 7 times) and a consequent increase in sensitivity (i.e. decrease in LOD) to trace metals in water have been observed (Nakamura et al 1996).

Single pulse and double pulse laser induced breakdown spectroscopy of trace elements in water has been done successfully by focusing a laser beam on a thin stream of liquid in jet form. Commercially available jets, for example Meinhard Nebulizer in mist mode as well as thin jet mode, have been successfully used. (Kumar et al 2003)
It has also been possible to measure different plasma parameters such as electron density and electron temperature using the Boltzmann's local thermal equilibrium (LTE) approximation and Stark broadening of the hydrogen alpha line. (Rai et al 2003)

In double pulse LIBS, the first laser ablates the liquid and the second laser finds the ablated plasma after a delay of a few microseconds. The second laser re-ionizes the rapidly cooling plasma created by the first laser, hence bringing a larger number of atomic species into an excited state. The effect of the time delay between the two laser pulses on the LIBS signal as well as on electron density has been studied. (Rai et al 2003)

An example of LIBS of Mg solution using Nd: YAG laser is presented here. Two laser pulses with a few microsecond inter-pulse delays have been used to produce and re-excite the plasma. The correlation between the electron density in the laser produced plasma at various time delays between the two lasers and the corresponding LIBS signal has been studied. The electron density is determined by using the Stark broadening of H_α line. In this study, a Meinhard Nebulizer has been used to create the thin liquid jet that introduces the liquid sample to the laser plasma. The Meinhard Nebulizer used for LIBS was originally developed for ICP (Inductively Coupled Plasma) applications, which can work both in very fine thin jet and mist modes depending on the requirement. The experimental setup to record the plasma emission of Mg in 2% acidic solution has been shown earlier in Figure 2.

In brief, a Q-switched, frequency doubled Nd: YAG laser that gives 532 nm radiation of 8 ns duration has been used to ablate the laser jet. To enhance the LIBS signal, another Q-switched, frequency doubled Nd:YAG laser is used to re-ablate and excite the laser produced plasma. The emission from the plasma was focused using a 20-cm focusing lens on the liquid jet/mist stream of a Nebulizer (Meinhard TR-30-C6) with a nozzle diameter of about 0.3 mm. The Meinhard Nebulizer was originally made for ICP measurements. It creates a mist and is optimized to work in conditions of 30 psi gas pressure with an argon gas flow rate of about 1000 ml/min and a liquid flow rate of 5.7 ml/min. But in the case of LIBS, we observed that the liquid sample solution flow rate of 3.5 ml/min and gas (argon) flow rate of 300 ml/min gave the best signal. Also, the same nebulizer has been used to produce a very fine and stable liquid jet at the liquid flow rate of 17.5 ml/min. A closed loop system has been used for circulating the solution. Emissions have been collected in the backward direction by coupling UV grade quartz lenses of focal length 50 cm and 10 cm lenses on the optical fiber bundle. The fiber bundle is a collection of 80 single fibers of 0.1 mm core diameter. The other end of the fiber is connected to a remote spectrograph equipped with a 2400- l/mm diffraction grating. The emission signal was recorded with an Intensified CCD Camera connected to the exit end of the spectrograph. The signal is finally processed and stored in a computer. One hundred shots of spectra are accumulated

for one spectrum. Thirty such spectra were recorded under the same conditions for a good statistical average.

Comparison of Double Pulse Applications of Laser Induced Breakdown Spectroscopy Signal in Mist and Thin Jet Modes:

It has been widely reported that two pulse LIBS is preferable to single pulse. Normally, after plasma is created by one pulse, it expands and the various species in their ionized state return to their neutral state by the electron-ion recombination process. The normal life-time of the plasma is of the order of 20-30 μs. Light emission from the plasma is the sum of the two distinct processes. The first process is recombination of free electrons and the free ions that contribute to the background emission. The second process is the emission from the atoms and ions due to the transition from one bound state to the other. The second process causes the line emission. After creation of the laser induced plasma, both kinds of emissions decay with time but at different decay rates. Background emissions, which are stronger in the initial few microseconds, decay faster than the emissions from the atoms and ions. The bound states emissions are used to characterize the elements present in the plasma/material. The purpose of the second laser pulse is to re-excite the plume created by the first laser pulse and, after a certain time delay, re-ionize the expanding plume. The delay timing between the lasers is selected in such a manner that the ionization of the concerned species (heavy metal atoms that expand little more slowly than the lighter atomic components of water and surrounding gas) is more than the unwanted species. As a result, the enhanced LIBS signal of the element of interest (Mg in the present case) is observed.

Variations of LIBS signal intensity of 20 ppm Mg in water in the jet mode and the mist mode using the Meinhard Nebulizer is shown in Figure 4. In Figure 4, the energy of two lasers is 150 mJ and 130 mJ respectively. The gate delay and gate width are 3μs and 10 μs respectively which are selected after initial optimization. It is evident from Figure 4 that as we increase the time delay between the two lasers, the LIBS signal increases in the case of jet mode and decreases in the case of mist mode. It is also clear from Figure 4 that on increasing the time delay between the lasers, the LIBS signal of Mg increases. The LIBS signal maximizes around a time delay of 3 μs and then starts decreasing. In mist mode, the behavior is different than in jet mode. In mist mode the LIBS signal goes on decreasing as the delay between the lasers is increased. In fact, the situation in mist is very different than in case of jet. In mist, the liquid particles are coming out in the form of fine droplets immersed in the gas (argon in the present case). When the laser beam is focused on the mist, each droplet behaves as a tiny lens that can focus the laser beam at random locations. Therefore, the second laser, which is supposed to re-excite the plasma plume created by the first pulse, may not find the right spot.

Comparison of Plasma Density in Double Pulse LIBS in Mist and Thin Jet modes:

The variation of electron density with the time delay between the lasers has been compared in Figure 5. To estimate the electron density, the H_α line at 656.2 nm is recorded in the same experimental conditions as were used to record the LIBS spectra of magnesium lines. Stark broadening of H_α line was used to estimate the electron temperature.

It is clear from the Figure 5, that initially when the time delay between the two lasers is zero, they behave as a single laser. The electron density in mist mode is higher than that in the jet mode due to the low ionization potential of the purging gas used in the mist mode. The laser plasma was generated in the argon atmosphere in the mist mode while it is generated in the water surrounded by the ambient gas in the jet mode. Also, for the jet mode on increasing the time delay, the electron density initially decreases then and then finally starts increasing. After the plasma generated by the first laser expands to a certain size, the second laser can effectively re-excite the plasma and increase the plasma density. Initially, when the time delay between the two lasers is zero, they work as a single laser with higher laser intensity. As the laser delay time equals 200 ns, LIBS signal intensity increases while the electron density goes down.

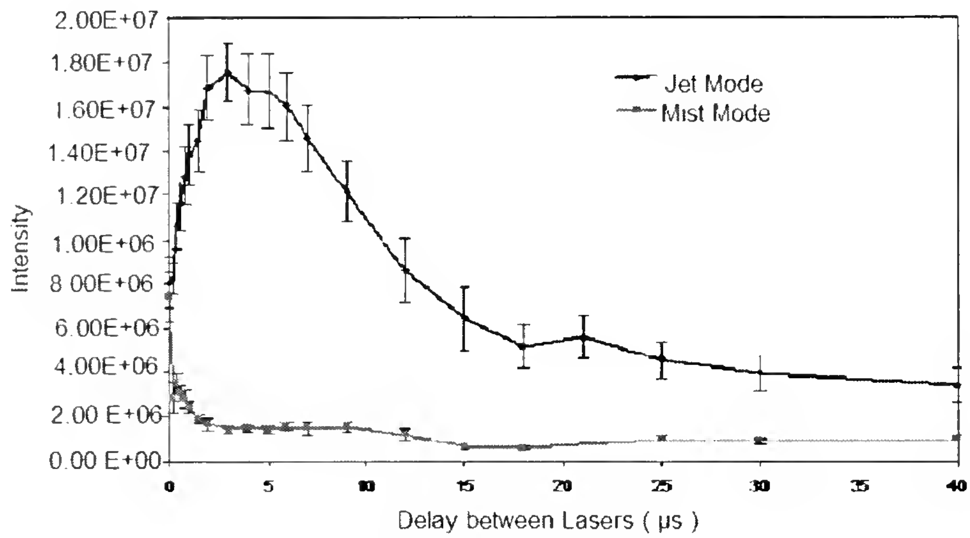


Figure 4. Comparison of applications of laser induced breakdown spectroscopy signal in jet and mist modes. A plot of intensity versus gate delay. [Mg 20ppm, 1μs gate delay, 10μs gate width, L1 = 140mJ (Big Sky), L2 = 160mJ (Surelite)].

The plasma density goes down because the plasma density generated by the first laser is lower than when the two laser pulses were together. It seems that initially the size of the plasma plume is so small and electron density is so high that the second laser is not able to reach the material and create its own ablation and plasma. Rather, it re-excites the plasma created by the first laser, causing an increase of the LIBS signal. With time, the size of the plasma plume becomes bigger and bigger, and the area of the plume of the plasma between the influence of the second laser becomes smaller and smaller. As a result, the density of the plasma is reduced. Beyond a critical time (delay = 1.5 μs), the plasma created by the first laser becomes diluted so that the second laser is able to penetrate the plume, creating the plasma from the material. As a result, the plasma density starts increasing and reaches a maximum. Further, on increasing the time delay, the influence of the first laser becomes weaker and weaker, and thus only the characteristic of the second laser plasma becomes prominent. By comparing double pulse LIBS signals in the jet mode

and mist mode, it is very obvious that jet mode is beneficial for the enhancement of the signal.

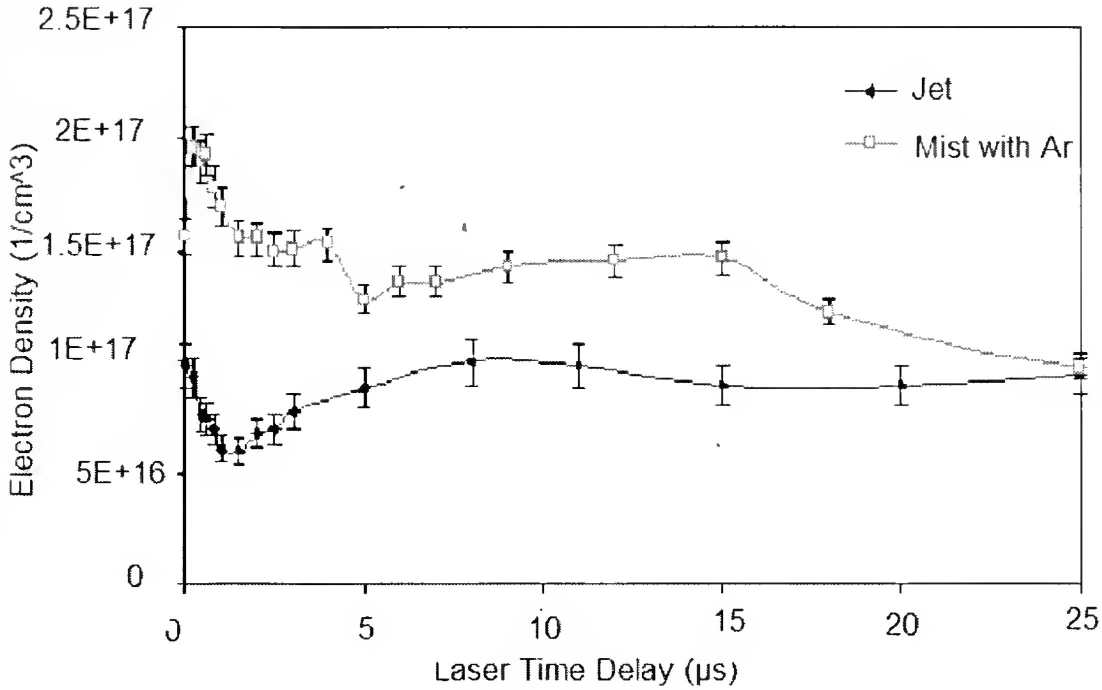


Figure 5. Variation of plasma density by changing the time delay between the two lasers in jet and mist modes of the nebulizer. [1us gate delay, 10 us gate width, L1 = 140 mJ (Big Sky), L2 = 160 mJ (Surelite)].

Applications of Laser Induced Breakdown Spectroscopy in Solid Media:

Applications of laser induced breakdown spectroscopy technique have successfully been used in monitoring the trace elements in different metals, glasses and both organic and inorganic media including bio-media. An example of detection of malignant cells using the LIBS spectroscopy is presented in this paper. In common practice, a tedious time consuming procedure is used in which the sample must be sent to a sophisticated laboratory and results are awaited for days. Laser induced breakdown spectroscopy (LIBS) is an equally good technique for the diagnosis of a solid medium or a mixed medium where solids and liquids coexist together. It has the same capability to provide real-time, multi-element detection of the trace element in the human body as in liquids and solids separately. In order to record the LIBS spectrum, an Echelle spectrometer has been used that can identify the different atomic and ionic lines in the spectral region 200 to 800 nm.

The correlation of human health to trace element analysis is widely reported in literature (Reinhold, J. G. et al., 1966). It is reported that the imbalance of trace elements in the human body can lead to several acute problems such as nervousness, cancer, tumors, etc (Pihl et al., 1980). Efforts have been made to use LIBS to identify different trace elements (for example iron, sodium and magnesium) present in the human blood, nails, hair and cancer cells (Kwiatek, W.M. et al., 1996). Concentration of the vital elements

is directly related to the health of the human body. Using this technique, the imbalance of trace elements in the body can be immediately detected and preventive measures can be adopted. Normally, patients must go to laboratories for pathological tests and wait to obtain the test results. By using LIBS technique, doctors and medical professionals can know immediately the levels of the trace elements in the body by just shining a spark of laser light on the nail or hair.

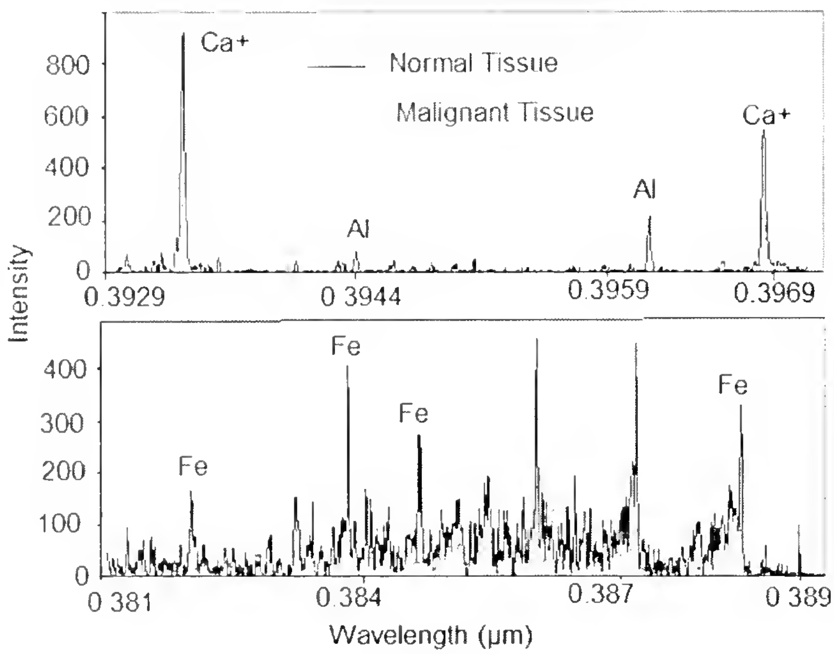


Figure 6. Applications of laser induced breakdown spectroscopy spectrum of malignant and normal tissue cells of dog liver.

It will be possible to explore these variations in the human body. During laser surgery, it will be possible to monitor the malignant portion of the body (Kumar et al 2004). Applications of laser induced breakdown spectroscopy spectrum of malignant and normal cells from a dog liver are depicted in Figure 6. The difference in the two spectra is very minute. However, when the ratio of intensity of different elemental lines with calcium is plotted (Figure 7) for malignant and normal cells, the distinction between the two is very evident.

It has been demonstrated that laser light can be used to extract blood from the human body. When the laser light is focused beneath the skin, it ablates the skin and provides a path for blood to exit. In this technique, no danger or infection is expected as opposed to the usage of conventional needles for blood collection. The whole procedure requires less than a minute, with no pain or physical damage to the patient. An attempt has been made to monitor trace elements in hair and nails. It has been reported that the nails and hair have similar structures and similar contents of trace elements. The normal growth rate of nails varies from 4 mm to 8 mm per month. For persons who have a deficiency of minerals, sampling different portions of the nail can monitor the effect of medication.

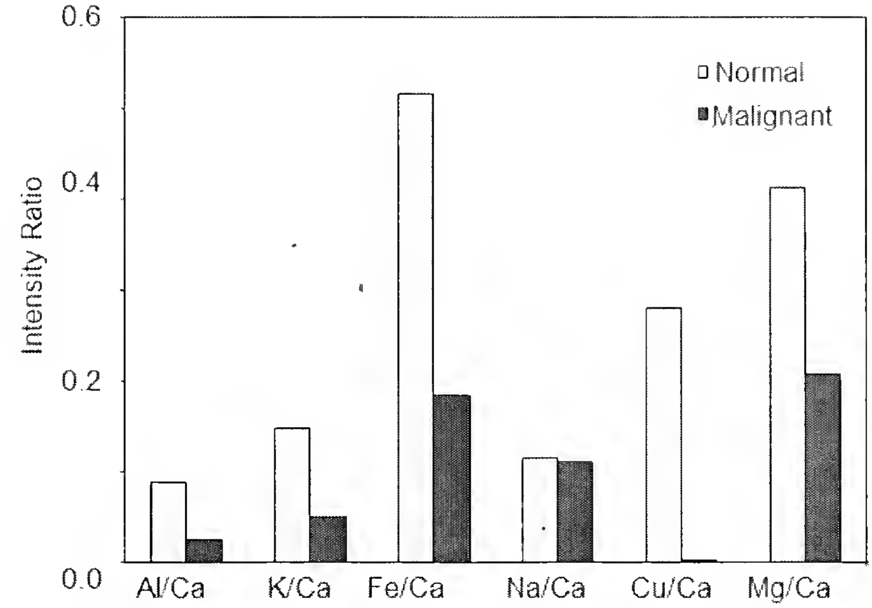


Figure 7. Plot of intensity ratio of applications of laser induced breakdown spectroscopy signal of different elements.

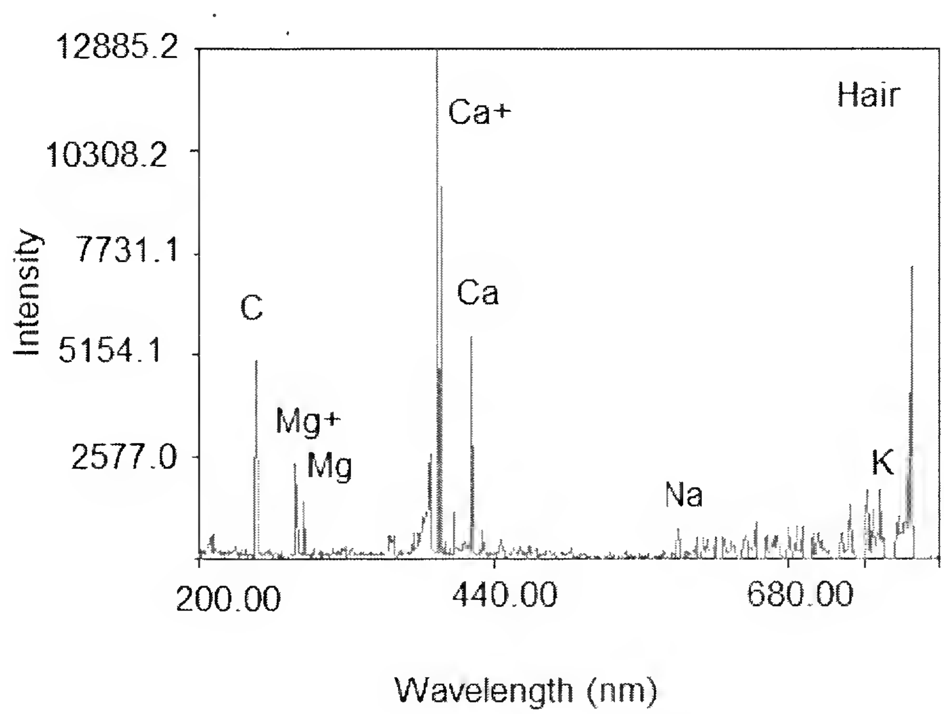


Figure 8. Spectrum of hair by laser induced breakdown spectroscopy.

Typical LIBS spectra of hair and nails obtained in the laboratory are shown respectively in Figures 8 and 9. It is very apparent from these figures that the intensity lines of magnesium, calcium and carbon are very strong whereas sodium and potassium are very weak in nails and hair spectra. It has been observed that lines of carbon and magnesium are stronger in nails as compared to hair as shown in Figures 8 and 9. The spectra shown in Figures 8 and 9 are recorded under similar experimental conditions at laser energy of

20 mJ, gate delay of 1 μs and gate width of 10 μs. From the spectra of hair and nails, one can observe the similarity of their structure. This similarity has been described elsewhere (Stevens B.J, 1983). Performing the LIBS experiment with nails is easier in comparison to hair because of the nails' large flat area. There is no need of special alignment of the laser focal point to the nail surface as in the case of hair. The nail is also firmer and more solid in comparison to hair. The LIBS is more pronounced for nails (approximately three times) in comparison to hair. In LIBS literature, this phenomenon is described as the matrix effect. Mass of the material ablated in the laser induced breakdown spectroscopy depends on the dielectric properties of the material. The trace elements detected by LIBS technique give different signal intensities in different solid matrices. This is commonly known as matrix effect.

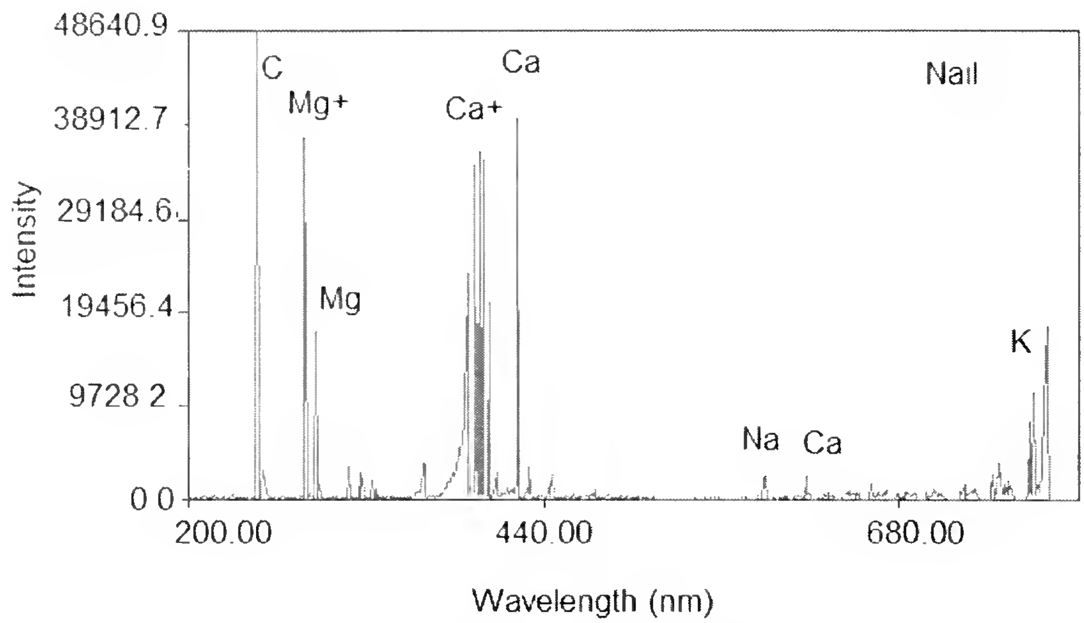


Figure 9. Spectrum of nails by laser induced breakdown spectroscopy.

Future applications of laser induced breakdown spectroscopy :

Laser induced breakdowns are a relatively new diagnostic tool, and it has been steadily gaining a lot of importance in different areas of technology and pure fundamental research. However, issues such as reproducibility of experimental results and production of a clean and controlled plasma using laser light still need to be worked on before LIBS becomes a standard industry tool. New additions such as use of femto-second laser (Margetic et al., 2003) and dual-pulse LIBS have taken LIBS into its next generation and have helped to resolve a number of issues being faced with this technique. There are huge potentials for LIBS in industry and the military. In the future, the development of more advanced detection systems and the cheap femto-second laser will bring this technology close to everybody's life in the coming years.

In summary, LIBS are a useful and very fast real-time technique to monitor trace elements in almost all phases of media. It has been observed that double pulse LIBS is

beneficial than single pulse as this gives a lower limit of detection (LOD) of trace elements. Also it has been established by this work that there is a correlation between the LIBS signal and the plasma density. It has been shown that LIBS technique can differentiate between normal and malignant tissue. It has also been shown that LIBS is equally effective in detecting trace elements in nails and hair samples.

ACKNOWLEDGEMENTS

The authors thank Provost Dr. Luther S. Williams and Dr. L. L. Burge, Jr., for their support. Thanks are also due to Dr. H. Jain, Chair Professor, Department of Physics, Lehigh University for his interest. We thankfully acknowledge Dr. Eugene Zakar, Program Manager, Army Research Laboratory, Adelphi, MD, for providing research funds to complete this work.

LITERATURE CITED

Cremers, D.A., L. Radziemski, T.R. Loree. 1984. Spectroscopic analysis of Liquids using Laser Spark. Applied Spectroscopy. 38: 721-726.

Hopps, H.C. 1974. The Biological Bases for using hair and nail for Analysis of Trace Elements. Trace Substances in Environmental Health VIII. Hemphill, D.D.ed. University of Missouri. Columbia. 7:71- 89

Kumar, Akshaya, F.Y. Yueh and J. P. Singh. 2003. Determination of trace elements in Liquid using Meinhard Nebulizer by Laser Induced Breadown Spectroscopy. Applied Optics. 42: 6040-6046.

Kumar, Akshaya, F.Y. Yueh and J. P. Singh. 2003. Characterization of Malignant Tissue Cells using Laser Induced Breakdown Spectroscopy. Applied Optics 43: 5399-5403.

Kwiatek, W.M., T. Drewnaik , M. Lekka, and A. Wajdowicz. 1996. Investigation of trace elements in cancer kidney tissues by SRIXE and PIXE. Nuclear Instruments and Methods in Physics Research Section B. 109: 284-288.

Margetic, V., T. Ban, F. Leis, K. Niemax, and R. Hergenroder. 2003. Hydrodynamic expansion of a Femtosecond Laser Produced Plasma. Spectrochimica Acta Part B. 58: 415-425.

Martin, M. and M. D. Cheng. 2000. Detection of Chromium Aerosol using Time Resolved Laser Induced Plasma Spectroscopy. Applied Spectroscopy. 54: 1279-1285.

Marquardt, B.J., S.R. Goode and S.M. Angel. 1996. In situ Determination of Lead in Paint by Laser Induced Breakdown Spectroscopy using a Fiber Optic Probe. Analytical Chemistry 68: 977-981.

Nakamura, S., Y. Ito and K. Sone et al. 1996. Determination of Iron Suspension in water by Laser Induced Breakdown Spectroscopy with Two Sequential Laser Pulses. Analytical Chemistry. 68: 2981-2986.

Pihl, R. O., H. Drake and F. Vrana. 1980. Department of Psychology, McGill University, Montreal, Quebec, Canada. Hair Analysis in Learning and Behavior Problems. *Hair, Trace Elements, and Human Illness.* Brown, A. C.; Crounse, R. G., (eds). Praeger Publications.USA.

Potter, J. L., G. D.Timmons, R . West, and A. A. Silvidi. 1974. Arginino succinic academia - The Hair Abnormality. American Journal of Diseases of Children. 127: 724-727.

Rai, V.N, A. K. Rai, F.Y. Yueh and J.P. Singh. 2003. Optical Emission from Laser Induced Breakdown Plasma of Solid and Liquid Samples in the presence of a Magnetic Field. Applied Optics 42: 2085-2093.

Rusak, D.A., B.C. Castle, B.W. Smith, and J.D. Winefordne. 1997. Critical Reviews in Analytical Chemistry, 27: 257-290.

Reinhold, J. G., G. A. Kfoury, M. A. Ghalambor and C. Jean. 1966. Zinc and Copper Concentrations in Hair of Iranian Villagers. The American Journal of Clinical Nutrition. 18: 294-300.

Saggese, S. and R. Greenwall. 1997. LIBS Fiber Optic Sensor for Subsurface Heavy Metal Detection. Proceeding of International Society for Optical Engineering (SPIE). 2836: 195-205.

Stevens, B. J. 1983. Determination of Aluminum, copper and Zinc in Human Hair. Atomic Spectroscopy. 4: 176-178.

Strain, W. H., L. T. Steadman, C. A. Lankau, Berliner W. P., Pories W. J. 1966. Analysis of Zinc Levels in Hair for the Diagnosis of Zinc Deficiency in Man. The Journal of Laboratory and Clinical Medicine. 68: 244-249.

Yueh F.Y., J.P. Singh and H. Zhang. 2000. 2066-2087. Elemental Analysis with Laser Induced Breakdown Spectroscopy. In Encyclopedia of Analytical Chemistry, John W-iley and Sons. Ltd, Chishester.

COMPARATIVE WATER RELATIONS OF THREE SYMPATRIC TERRESTRIAL SLUGS: (STYLOMMATOPHORA: AGRIOLIMACIDAE, LIMACIDAE, AND PHILOMYCIDAE)

Jody M. Thompson[1], Arthur G. Appel[2], Jeff L. Sibley[3], Gary J. Keever[3], and Wheeler G. Foshee III[3]

[1]*Alabama Department of Conservation and Natural Resources, State Lands Division, Natural Heritage Section, 64 North Union Street, Montgomery, AL 36130, USA*
[2]*Department of Entomology and Plant Pathology, Auburn University, 301 Funchess Hall, Auburn, AL 36849-5413, USA*
[3]*Department of Horticulture, Auburn University, 101 Funchess Hall, Auburn, AL 36849, USA*

Correspondence: Appel, Arthur (appelag@auburn.edu)

ABSTRACT

Water relations of three sympatric species of terrestrial slugs found in the southeastern United States were examined in laboratory experiments. Fully hydrated slugs were desiccated at 0-2% RH and 30°C. Slugs were weighed and mortality was recorded every 2 h for 12 h and at 24 h. Initial mass, percentage of total body water (% TBW), cuticular permeability (CP), water loss rate (WL), hour of death (HR), and % TBW lost at hour of death (% LHR) were determined. Initial masses ranged from 0.08 g for *Deroceras laeve* to 2.87 g for *Philomycus carolinianus*. Water content (% TBW) was ≈85.69% and did not differ among species. *Philomycus carolinianus* was the most desiccation tolerant having the lowest CP (210.20 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$), lowest WL (57.87 $mg\ g^{-1}$), longest HR (23.06), and highest % LHR (89.13%). *Lehmannia valentiana* was more desiccation tolerant than *D. laeve* having a lower WL (218.25 $mg\ g^{-1}$ compared with 322.97 $mg\ g^{-1}$) and greater HR (5.26 compared with 2.5). Although *P. carolinianus* was more tolerant to desiccation, it is distributed primarily in relatively stable undisturbed habitats. Additionally, *L. valentiana,* which is found mainly in synanthropic habitats, and *D. laeve*, which can be found in both disturbed and undisturbed habitats were less tolerant of desiccation.

INTRODUCTION

Terrestrial slugs are one of the most successful groups of mollusks, inhabiting nearly every type of terrestrial ecosystem across the globe, except Antarctica. Excluding semi-slugs, there are approximately 550 - 600 spp. of terrestrial slugs in more than fifteen families worldwide (D. G. Robinson, USDA, pers. com.). Even though slugs are closely related to snails, loss of the shell has lessened the need for calcium salts, so slugs can live in a wider range of habitats than most snails, including low calcium environments such as

agricultural fields and suburban gardens (South, 1992). However, without a shell, slugs are more susceptible to desiccation. Slugs compensate for their poor control over water loss by having increased mobility and burrowing more easily into soil as well as having greater tolerance to body water losses than do snails (Machin, 1975).

Terrestrial slugs and snails are well known for their mucus. Mucus facilitates locomotion and mating, and can be used as a defensive secretion (Denny, 1983; Deyrup-Olsen et al., 1983). Slugs lose water rapidly through the production of mucus as well as from their moist integument and lung surfaces (Prior, 1985). Machin (1972) found that the mucus normally maintained on a snail epidermis has no significant water-proofing properties. The chemical and physical characteristics of secreted mucus can vary depending on its immediate physiological role and possibly between species (Deyrup-Olsen et al., 1983; Skingsley et al., 2000).

There are eight families of true terrestrial slugs (Reise et al., 2000; Turgeon et al. 1998) in North America. With few exceptions, all species of economic importance in North America are introduced, many from northern Europe (South, 1992). Slugs are usually considered pests because many species damage agricultural crops, are vectors of plant and animal diseases, and compete with native species (South, 1992; Rollo, 1983). In urban and agricultural areas, exotic slugs such as *Deroceras reticulatum* (Müller) destroy crops and garden plants. While many slug species are pests, this is not true in all situations; in undisturbed habitats, native slugs are usually benign. Native slugs such as *Philomycus* spp. and *Pallifera* spp. can be found in and under rotting logs and on trees, generally avoiding open habitats.

Deroceras laeve (Müller) is a significant pest of crops such as corn and soybeans and has a Holarctic distribution (Barker, 2002; Kerney and Cameron, 1979). Kerney and Cameron (1979) described the natural European habitat of *D. laeve* as relatively wet (fens, river-banks, moist woodlands, and meadows); this is also true for its North American habitats. *Deroceras laeve* occurs in wild areas and cultivated habitats of almost every description, including urban areas (Chichester and Getz, 1969).

Lehmannia valentiana (Férussac) is native to the Iberian Peninsula but has been spread by commerce throughout the world and is considered a pest species wherever it has been introduced (Kerney and Cameron, 1979; South, 1992). This species is found throughout North America, principally in synanthropic habitats (Chichester and Getz, 1969; Forsyth, 2001). *Lehmannia valentiana* is one of the most common slug species found in the eastern United States and is most often associated with greenhouses in the northeast (Chichester and Getz, 1969; D.G. Robinson, USDA, pers. com.). In the southeastern United States (such as southern Alabama), *L. valentiana* is found under boards, concrete slabs, and rotting wood and apparently does not require the protection of greenhouses (pers. obs.). Waldén (1961) reported that *L. valentiana* is naturalized in California and Chile. It is likely that future slug surveys will find additional naturalized populations of *L. valentiana* outside of greenhouses.

Philomycus carolinianus (Bosc.) is native to eastern North America, is found in undisturbed wooded habitats throughout the eastern United States, and has not been

reported as a pest (Hubricht, 1985; D.G. Robinson, pers. com.). In a distribution study, Chichester and Getz (1969) rarely found *P. carolinianus* outside of wooded habitats.

This study examined water relations of three sympatric species of terrestrial slugs found in the southeastern United States: *D. laeve* (family Agriolimacidae), *L. valentiana* (family Limacidae), and *P. carolinianus* (family Philomycidae). The purpose of this study was to compare the environmental adaptations and tolerances of native and exotic species of terrestrial slugs by determining several aspects of their water relations and desiccation tolerance. We hypothesize that invasive, pest species of slugs have either a lower rate of desiccation or lower sensitivity to water loss than native species. Although there have been several studies on slug water relations, most studies that incorporate cuticular permeability involve terrestrial arthropods. Cuticular permeability is often one of the most important factors that limit water loss in terrestrial arthropods and has been used as a measure of environmental adaptiveness for many arthropod species (Edney, 1977; Hadley, 1994). Understanding slug adaptiveness and desiccation tolerance may lead to better predictions of potential distributions of invasive species, more effective management of pest slugs, and conservation of native species.

MATERIALS AND METHODS

Lehmannia valentiana and *Deroceras laeve* were collected from the grounds of the Auburn University Paterson Greenhouse Complex, Auburn, Lee County, Alabama, USA. Slugs were removed from water valve covers, from cultured hosta, and from under loose boards. *Philomycus carolinianus* were collected from a forested rural area of Macon County, Alabama, USA within and under rotting logs and from the bark of trees (*Magnolia virginiana* L. and *Liquidambar styraciflua* L.). All slugs were collected during daylight hours in June and July 2004.

In the laboratory, slugs were separated by species and transferred into clear plastic containers (12.5 x 18.0 x 7.0 cm) lined with moist paper towels. Paper towel liners were changed twice weekly and sprayed with tap water daily to maintain moisture. Slugs were fed commercially grown lettuce, which was eaten readily by *D. laeve* and *L. valentiana,* but not *P. carolinianus*. All slugs were maintained at room temperature (22 ± 2°C) and starved for a minimum of 24 h prior to testing.

Water Relations

Initial mass of 20 fully hydrated slugs of each species was determined gravimetrically with a digital balance (0.01 mg sensitivity). Live slugs were transferred with soft forceps to individual black fiberglass screen bags (6 x 8 x 4 cm). Screen bags prevented slugs from anchoring the entire surface of their foot against an impermeable surface; thus all parts of the slugs were exposed to desiccating conditions as could occur while a slug was climbing. After the slugs were weighed, the bags containing the slugs were placed on a porcelain ring in an 11 L desiccator with ≈2.3 kg of anhydrous $CaSO_4$ that was used to maintain the air inside the desiccator at 0-2% RH. The desiccant was dried at 60 ± 1°C for a minimum of 24 h to remove all water before being cooled and placed in the desiccator. The desiccator, containing up to 20 slugs, was placed in an incubator

maintained at 30 ± 1°C, a common summer temperature in Alabama as well as other parts of the southeastern United States. The mass of each slug was measured after 0, 2, 4, 6, 8, 10, 12, and 24 h of desiccation. Mass loss between weighings was assumed to be a result of water loss. The condition of each slug (live or dead) was recorded at each weighing interval. Dry mass was determined by placing 24 h-desiccated specimens in individual 30 ml glass shell vials and drying them at 60 ± 1°C for a minimum of 48 h or until two successive weighings did not differ by >1%. Percentage of total body water (% TBW) was calculated using the following equation:

$$\%\ TBW = [(\text{initial mass} - \text{dry mass}) / \text{initial mass}] * 100.$$

Cuticular permeability was calculated as μg of water lost per cm^2 per h per unit saturation deficit (mmHg) in the desiccator chamber. The saturation deficit in the desiccator was 31.824 mmHg (0% RH and 30°C). No distinction was made between the cuticular permeability of the differing body regions (e.g., mantle or foot) because Machin (1975) found that water is lost similarly from all exposed surfaces of the body of a terrestrial pulmonate body at humidities < 99.5% at 20°C. Surface area for the cuticular permeability calculation was estimated for each specimen using Meeh's formula (Meeh, 1897):

$$S = 12M^{2/3},$$

where S is surface area (cm^2) and M is initial mass (g). Water loss rates (WL) were calculated as the total mg of water lost per g of initial mass over time for the first 2 h of desiccation.

Data Analysis

Treatments were arranged in a randomized complete block design and replicated three times. Analysis of variance (ANOVA, PROC GLM, SAS Institute, 1999) was used to detect differences in initial mass, % TBW, CP, WL, hour of death (HR), and percentage of total body water lost at hour of death (% LHR) among species. Means were separated by the Waller-Duncan K ratio t-test (SAS Institute, 1999). Cuticular permeability values were regressed against initial mass to test if Meeh's formula provided an unbiased estimation of surface area. Percentage of initial mass loss, % total body water loss, and mg g^{-1} water loss over time were examined using a two-parameter exponential equation of the form $y = a\,(1 - e^{-bx})$, where x is time (h), y is loss, a is asymptotic maximum water loss, and b is the slope. A significance level of $P \leq 0.05$ was used in all statistical tests. Data are expressed as means ± SE.

RESULTS

Mean initial mass ranged from 0.08 g for *Deroceras laeve* to 2.87 g for *Philomycus carolinianus* (Table 1). There was no difference ($P > 0.05$) in initial mass between *D. laeve* and *L. valentiana*; however, initial mass of *P. carolinianus* was significantly greater than that for the other species ($F = 140.95$; df = 2; $P < 0.0001$). Mean %TBW ranged from 85.23% for *P. carolinianus* to 86.58% for *D. laeve* (Table 1); there were no significant differences among the species ($P = 0.6439$).

Using linear regression, cuticular permeability values (using Meeh's formula to calculate body surface area) were not significantly ($P > 0.05$) related to initial body mass for all three species. This indicates that CP was not affected by initial mass and that Meeh's

formula was an unbiased estimate of surface area for these slugs. The mean CP of the slugs ranged from 210.20 μg cm^{-2} h^{-1} $mmHg^{-1}$ for *P. carolinianus* to 378.67 μg cm^{-2} h^{-1} $mmHg^{-1}$ for *L. valentiana* (Table 1). There was no difference ($P > 0.05$) in CP between *D. laeve* and *L. valentiana*, however; the CP of *P. carolinianus* was significantly less than in the other species ($F = 51.81$; df = 2; $P < 0.0001$). The mean WL of the slugs after 2 h of desiccation ranged from 57.87 mg g^{-1} for *P. carolinianus* to 322.97 mg g^{-1} for *D. laeve* (Table 1). There was a significant difference in WL among the three slug species ($F = 146.50$; df = 2; $P < 0.0001$). Mean hour of death (HR) was also significantly different among the three slug species ($F = 393.94$; df = 2; $P < 0.0001$), at 2.50, 5.26, and 23.06 h for *D. laeve*, *L. valentiana*, and *P. carolinianus*, respectively (Table 1). Mean %LHR for *D. laeve* and *L. valentiana* was 67.77 and 64.45%, respectively, and was not different, but %LHR for *P. carolinianus* was significantly greater than that for the other slugs ($F = 51.81$; df = 2; $P < 0.0001$) at 89.13%.

Regression coefficient *a*, asymptotic maximum water loss, was significantly different for *D. laeve*, *L. valentiana*, and *P. carolinianus* for % initial mass loss and mg g^{-1} loss; there was no overlap in coefficients (means ± 2 SE) (Table 2). The regression coefficient *a* was the same for *D. laeve* and *L. valentiana* for % total body water loss but significantly greater for *P. carolinianus*. The regression coefficient *b* (slope) was different for each species, with *D. laeve* being the greatest (0.71) and *P. carolinianus* being the smallest (0.065) for all regressions. The greater the value for the slope, the more rapidly the slug lost water to reach maximum water loss. After 2 h of desiccation, *D. laeve*, *L. valentiana*, and *P. carolinianus* lost 74.53, 49.47, and 12.95% of total body water (Fig. 1), respectively. After 24 h of desiccation, *D. laeve*, *L. valentiana*, and *P. carolinianus* lost 98.52, 97.48, and 83.99% total body water, respectively (Fig. 1).

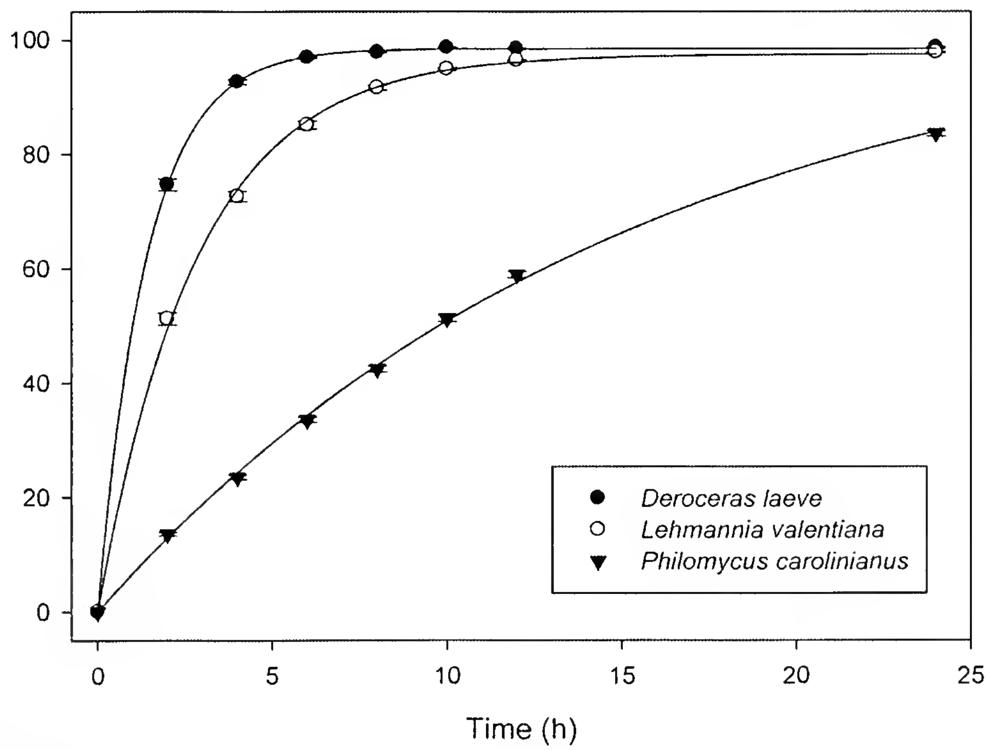


Figure 1. Percentage of total body water loss over time (h) for three slug species.

Table 1. Mean (± SE) initial mass, % total body water (%TBW), cuticular permeability (CP), water loss rates at 2 h (WL), hour of death (HR), and percentage of total body water loss at hour of death (% LHR) of three slug species

Species	N	Initial mass (g) (min-max)	% TBW (min-max)	CP ($\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$) (min-max)	WL ($mg\ g^{-1}$) (min-max)	HR (min-max)	% LHR (min-max)
Deroceras laeve	20	0.08 ± 0.01a	86.58 ± 1.16a	348.00 ± 14.86a	322.97 ± 14.19a	2.50 ± 0.20a	67.77 ± 0.50a
		(0.03-0.17)	(68.84-96.85)	(216.20-462.52)	(226.71-457.83)	(2-4)	(66.52-71.54)
Lehmannia valentiana	20	0.34 ± 0.04a	85.27 ± 0.48a	378.67 ± 16.76a	218.25 ± 13.08b	5.26 ± 0.31b	64.45 ± 0.97a
		(0.14-0.83)	(81.11-88.57)	(223.91-499.22)	(101.70-298.05)	(4-8)	(60.54-72.93)
Philomycus carolinianus	20	2.87 ± 0.22b	85.23 ± 0.70a	210.20 ± 10.24b	57.87 ± 3.20c	23.06 ± 0.94c	89.13 ± 3.23b
		(1.26-5.05)	(75.69-88.99)	(129.31-293.76)	(37.27-82.57)	(8-24)	(37.49-92.35)

Means followed by the same letter within a column are not significantly different ($P > 0.05$); Waller-Duncan K-ratio t test.

Table 2. Regression coefficients of percentage of initial mass loss, percentage of total body water (% TBW) loss, and mg g^{-1} water loss (WL) over time (h) of three slug species[1]

Regression	Species	*a*	*b*	*F*	*P*	R^2
Percentage Mass Loss	*Deroceras laeve*	85.29 ± 0.078	0.71 ± 0.0042	212094.88	<0.0001	0.999
	Lehmannia valentiana	83.14 ± 0.49	0.35 ± 0.0083	9082.21	<0.0001	0.999
	Philomycus carolinianus	90.73 ± 2.21	0.065 ± 0.0026	7091.66	<0.0001	0.999
% TBW Loss	*Deroceras laeve*	97.52 ± 0.12	0.71 ± 0.0058	113077.57	<0.0001	0.999
	Lehmannia valentiana	97.50 ± 0.58	0.35 ± 0.0083	9020.83	<0.0001	0.999
	Philomycus carolinianus	106.41 ± 2.60	0.065 ± 0.0026	7090.19	<0.0001	0.999
WL (mg g^{-1})	*Deroceras laeve*	852.45 ± 1.05	0.71 ± 0.0057	118851.07	<0.0001	0.999
	Lehmannia valentiana	829.60 ± 4.98	0.36 ± 0.0085	8719.89	<0.0001	0.999
	Philomycus carolinianus	907.36 ± 22.11	0.065 ± 0.0026	7105.55	<0.0001	0.999

[1] Power function regression coefficients (mean ± SE) determined using a two-parameter exponential equation [$y = a(1 - e^{-bx})$], x = time (h); y = loss; a = asymptotic maximum water loss; b = slope; $P < 0.05$.

DISCUSSION

Initial mass was greatest for *Philomycus carolinianus*, followed by *Lehmannia valentiana* and *Deroceras laeve*. Initial mass for *L. valentiana* and *D. laeve* was not significantly different. There was no significant difference in %TBW among the three species of slugs; % TBW varied <2%. These water contents are similar to those reported by Machin (1975) for *Arion ater* L., Arionidae (87.5% and 86.3%) and by Prior et al. (1983) for *Limax maximus* L., Limacidae (86.2%). For slugs, water contents are not a stable characteristic for species separations (South, 1992). Similarities in anatomy and physiology as well as general microhabitat requirements (e.g., relatively high moisture) and similar rearing conditions probably account at least partially for the similar water contents.

Cuticular permeability was lowest for *P. carolinianus* indicating that water was lost through the integument of this species more slowly than from the other species. The maximum CP value recorded in this study for *P. carolinianus* is lower than the mean CP value for both *D. laeve* and *L. valentiana*. Higher CP values are generally associated with organisms living in hygric environments whereas lower rates are associated with organisms living in more xeric conditions (Appel and Tanley, 1999). The range of CP for arthropods is from 0.75 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ in desert tenebrionid beetles (Nicholson, Louw, and Edney, 1984) to 270 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ in a tropical centipede (Mead-Briggs, 1956). Machin and Lampert (1985, 1987) demonstrated that permeability is greater in cuticles containing more water. Although water content of the cuticle was not measured in this study, it likely contains a high percentage of water and its surface is obviously moist. Machin (1975) reported that all soft parts of pulmonates contain appreciable amounts of water. Therefore a higher CP is expected for slugs than for lipid-coated arthropods. The CP values for *D. laeve* and *L. valentiana* are greater than those reported for hygric arthropods.

Water loss rates were significantly different among all three species. *Philomycus carolinianus* had the lowest, followed by *L. valentiana* and *D. laeve*. The snails *Otala lactea* (Müller) and *Cornu aspersum* (Müller) can reduce the rate of evaporative water loss from their mantles (Machin, 1966, 1972, 1974). Control of evaporative water loss was due to an increase in the concentration of K^+ and Cl^- in the apical cytoplasm of the mantle collar cells (Newell and Appleton, 1979). If terrestrial slugs can also regulate water loss through their mantles, this could explain the lower CP and WL values of *P. carolinianus* because the mantle covers the entire dorsum of this species whereas the mantles of *L. valentiana* and *D. laeve* cover only the anterior dorsum. Additionally, the mantles of *D. laeve* and *L. valentiana* are of similar size in relation to their body size. The similar mantle size to body size relationship could help to explain the similar CP values of *D. laeve* and *L. valentiana*. Machin (1972) determined the mantle CP of *O. lactea* to be 16 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$, which is more than 10-fold lower value than the CP values of a slug's entire body.

The hour of death (HR) for each of the three slug species differed significantly. *Deroceras laeve* died the fastest (2.5 h), *L. valentiana* took more than twice as long to die (5.3 h) as *D. laeve*, and *P. carolinianus* survived more than four times longer (23.1 h) than *L. valentiana*. Although the HR and % TBW lost at death values for *P. carolinianus* may be somewhat inflated because no observations were recorded between 12 and 24 h, there were

several slugs alive at 24 h and most of the dead slugs were still pliable. These observations indicate that mortality did occur at or very near 24 h of desiccation. Machin (1975) listed a variety of slug species from various authorities capable of surviving body mass losses of 60 to 80%. *Deroceras laeve* and *L. valentiana* lost approximately the same amount of water at their respective times of death (ca. 65%) even though their times of death were different. *Philomycus carolinianus* lost a significantly greater amount of water at death. Had the desiccation rate of *D. laeve* and *L. valentiana* been slower, at a lower temperature or higher RH, it is likely that their hour of death and % TBW losses would have been greater due to reduced stress on the slugs.

At 0 % RH and 30°C, *D. laeve* lost > 60% of its initial mass as water in the first 2 h of desiccation and > 85% by hour 24. *Deroceras laeve* had the highest rate of water loss as determined by having the greatest slope of regression. *Lehmannia valentiana* lost > 60% of its initial mass as water in the first 4 h and > 80% by hour 24. *Deroceras laeve* and *L. valentiana* showed their greatest rate of water loss within the first 6 h of desiccation, after which there was little additional water loss up to 24 h. *Philomycus carolinianus* did not have > 60% water loss until after hour 12 and showed a steady rate of loss throughout the course of the study. *Philomycus carolinianus* had the lowest rate of water loss as determined by having the smallest slope of regression due to probably having a lower CP and a lower surface area to volume ratio.

During collections for this study, *P. carolinianus* was most abundant in undisturbed wooded areas. *Lehmannia valentiana* was restricted to urbanized areas whereas *D. laeve* was found in both wooded and urbanized areas. These habitat associations agree with those reported by Chichester and Getz (1969). Even though *P. carolinianus* is more desiccation tolerant than *L. valentiana*, the two species seldom co-occur. An adequate food source is one factor that may limit the success of *P. carolinianus* in habitats preferred by *L. valentiana*. Various fungi are believed to be the principal food source of *P. carolinianus* (Branson, 1980). In disturbed environments, fungal diversity is reduced compared with undisturbed wooded environments (McDonnell et al., 1997). *Philomycus carolinianus* did not consume any of the lettuce provided prior to the start of this study. Dimelow (1962) found that a similar species, *Philomycus flexuolaris* Raf., did not accept any of the vegetative parts of the flowering plants offered but fed on lichens, fungi, and carrot slices. In contrast, *D. laeve* and *L. valentiana* are opportunistic consumers of fresh and decaying organic materials. Additionally, the inability to acclimate to relatively sudden temperature changes could reduce the capability of *P. carolinianus* to inhabit synanthropic areas. In a study on oxygen consumption and temperature preference of *Limax maximus* L. and *P. carolinianus*, Rising and Armitage (1969) determined that *P. carolinianus* did not acclimate readily to changes in temperature but showed a preferred optimal temperature range of 22-25°C. Forest canopy cover reduces temperature fluctuations contributing to greater environmental stability compared to unforested areas (Ovington, 1965).

When slug species were collected, *D. laeve* was more abundant in well-irrigated habitats compared with relatively drier areas where *L. valentiana* was abundant. One possible explanation for the greater success of *L. valentiana* in drier habitats compared to *D. laeve* is its tendency to form aggregations as is described for other slug species (South, 1992). Aggregations or "huddling" may help to minimize water loss (Cook, 1981).

Deroceras laeve does not form aggregations and is often aggressive towards conspecifics in high-density populations (South, 1992). However, Hommay et al. (2001) noticed aggressive behavior and even cannibalism in *L. valentiana*. In contrast, *P. carolinianus* did not form aggregations during this study.

Results of this study indicate that *P. carolinianus* is the most desiccation tolerant of the three slug species examined. Greater initial mass, lower CP, lower rate of WL, longer HR, and greater % TBW loss at death for *P. carolinianus* indicate that it loses body water under desiccation conditions much more slowly and tolerates greater body water losses than *D. laeve* and *L. valentiana*. The findings of Machin (1966, 1972, 1974) with regards to the water regulating ability of the mantle further support our conclusions. *Philomycus carolinianus* is physiologically more suitable to the more arid conditions of an urban and suburban habitat than either *D. laeve* or *L. valentiana*. However, *P. carolinianus* does not seem suited to the less stable temperature gradients and lack of fungal food sources associated with urban and suburban habitats. Getz (1959) found that *D. laeve* is tolerant of at least subfreezing temperatures. Additionally, *D. laeve* can be found in a greater range of habitat types than either *L. valentiana* or *P. carolinianus*. The data from this study also support the conclusion that *L. valentiana* is more desiccation tolerant than *D. laeve*. *Lehmannia valentiana* and *D. laeve* showed no difference in CP but, *L. valentiana* had a lower rate of WL and survived longer in desiccating conditions. The greater WL rate and shorter survival time probably have an influence on the habitat choices observed for *D. laeve*.

Much of what is known about terrestrial gastropod ecology is from casual observations only. Water relations represent a basic component of slug habitat preferences. A more integrative understanding of slug ecology and physiology will provide information on the potential of invasive species to become pests. Similarly, conservation of native species should involve studies of habitat tolerances, food preference, reproductive ecology, and community interactions. Researchers should also consider experiments on water relations that incorporate temperature gradients.

ACKNOWLEDGEMENTS

We thank Dr. Tim Pearce, Curator of Mollusks at the Carnegie Museum of Natural History and Dr. Mike Gangloff, Invertebrate Collections Manager at the Auburn University Natural History Museum and Learning Center for their comments and suggestions. We also thank Auburn University, the Department of Horticulture, and the Department of Entomology and Plant Pathology for their support.

REFERENCES

Appel, A. G., and M. J. Tanley. 1999. Water composition and loss by body color and form mutants of the German cockroach (Dictyoptera: Blattellidae). *Comparative Biochemistry and Physiology*, 122A: 415-420.

Barker, G. M. 2002. *Molluscs as Crop Pests*. CABI Publishing, Wallingford.

Branson, B. A. 1980. The recent Gastropoda of Oklahoma, Part VIII. The slug families Limacidae, Arionidae, Veronicellidae, and Philomycidae. *Proceedings of the Oklahoma Academy of Science*, 60: 29-35.

Chichester, L. F., and L. L. Getz. 1969. The zoogeography and ecology of Arionid and Limacid slugs introduced into northeastern North America. *Malacologia*, 7: 313-346.

Cook, A. 1981. Huddling and the control of water loss by the slug *Limax pseudoflavus* Evans. *Animal Behaviour*, 29: 289-298.

Denny, M. 1983. Molecular biomechanics of molluscan mucous secretions. In: *The Mollusca,* 1: *Metabolic Biochemistry and Molecular Biomechanics* (K. M. Wilbur and P. W. Hochachka, eds), 431-465. Academic Press, New York.

Deyrup-Olsen, I., D. L. Luchtel, and A. W. Martin. 1983. Components of mucus of terrestrial slugs (Gastropoda). *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 245: 448-452.

Dimelow, E. J. 1962. On the biology of some mollusks from a Nova Scotia deciduous wood. *The Nautilus*, 76: 49-51.

Edney, E. B. 1977. *Water Balance in Land Arthropods*. Springer-Verlag, New York.

Forsyth, R. G. 2001. First records of the European land slug *Lehmannia valentiana* in British Columbia, Canada. *The Festivus*, 33: 75-78.

Forsyth, R. G. 2004. *Land Snails of British Columbia.* Royal British Columbia Museum, Victoria.

Getz, L. L. 1959. Notes on the ecology of slugs: *Arion circumscriptus*, *Deroceras reticulatum*, and *D. laeve*. *American Midland Naturalist*, 61: 485-498.

Hadley, N. F. 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, San Diego.

Hommay, G., J. C. Kienlen, C. Gertz, and A. Hill. 2001. Growth and reproduction of the slug *Limax valentianus* Férrusac in experimental conditions. *Journal of Molluscan Studies*, 67: 191-207.

Hubricht, L. 1985. The Distributions of the Native Land Mollusks of the Eastern United States. *Fieldiana*, new series no. 24. Field Museum of Natural History, Chicago, pp. 191.

Kerney, M. P., and R. A. D. Cameron. 1979. *A Field Guide to the Land Snails of Britain and North-West Europe*. Collins, London.

Machin, J. 1966. The evaporation of water from *Helix aspersa*. IV. Loss from the mantle of the inactive snail. *Journal of Experimental Biology*, 45: 269-278.

Machin, J. 1972. Water exchange in the mantle of a terrestrial snail during periods of reduced evaporative loss. *Journal of Experimental Biology*, 57: 103-111.

Machin, J. 1974. Osmotic gradients across snail epidermis: Evidence for a water barrier. *Science*, new series 183, no. 4126: 759-760.

Machin, J. 1975. Water relationships. In: *Pulmonates* (V. Fretter and J. Peake, eds), 1: 105-163. Academic Press, London.

Machin, J., and G. J. Lampert. 1985. A passive two-layer permeability-water content model for *Periplaneta* cuticle. *Journal of Experimental Biology*, 117: 171-179.

Machin, J., and G. J. Lampert. 1987. An improved water content model for *Periplaneta* cuticle. *Journal of Insect Physiology*, 33: 647-655.

McDonnell, M. J., S. T. A Pickett, P. Groffman, P. Bohlen, R. V. Pouyat, W. C. Zipperer, R. W. Parmelee, M. M. Carreiro, and K. Medley. 1997. Ecosystem processes along an urban-to-rural gradient. *Urban Ecosystems*, 1: 21-36.

Mead-Briggs, A. R. 1956. The effect of temperature upon the permeability to water of arthropod cuticles. *Journal of Experimental Biology*, 33: 737-749.

Meeh, K. 1897. Oberflächenmessungen des menschlichen Körpers. *Zeitschrift für Biologie*, 15: 425-458.

Newell, P. F., and T. C. Appleton. 1979. Aestivating snails – The physiology of water regulation in the mantle of the terrestrial pulmonate *Otala lactea*. *Malacologia*, 18: 575-581.

Nicholson, S. W., G. N. Louw, and E. B. Edney. 1984. Use of a ventilated capsule and tritiated water to measure evaporative water losses in a tenebrionid beetle. *Journal of Experimental Biology*, 108: 477-481.

Ovington, J. D. 1965. *Woodlands*. English University Press, London.

Prior, D. J., M. Hume, D. Vargas, and S. D. Hess. 1983. Physiological and behavioural aspects of water balance and respiratory function in the terrestrial slug, *Limax maximus*. *Journal of Experimental Biology*, 104: 111-127.

Prior, D. J. 1985. Water-regulatory behaviour in terrestrial gastropods. *Biological Review*, 60: 403-424.

Reise, H., J. M. C. Hutchinson, R. G. Forsyth, and T. J. Forsyth. 2000. The ecology and rapid spread of the terrestrial slug *Boettgerilla pallens* in Europe with reference to its recent discovery in North America. *The Veliger*, 43: 313-318.

Rising, T. L., and K. B. Armitage. 1969. Acclimation to temperature by the terrestrial gastropods, *Limax maximus* and *Philomycus carolinianus*: oxygen consumption and temperature preference. *Comparative Biochemistry and Physiology*, 30: 1091-1114.

Rollo, C. D. 1983. Consequences of competition on the reproduction and mortality of three species of terrestrial slugs. *Researches on Population Ecology*, 25: 20-43.

SAS Institute. 1999. *User's manual, version 8.0*. SAS Institute, Cary, NC.

Skingsley, D. R., A. J. White, and A. Weston. 2000. Analysis of pulmonate mucus by infrared spectroscopy. *Journal of Molluscan Studies*, 66: 363-371.

South, A. 1992. Terrestrial Slugs: Biology, Ecology, and Control. Chapman and Hall, London.

Turgeon, D. D., J. F. Quinn Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg Jr., W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. G. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. *Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks*. 2nd Edition. American Fisheries Society.

Waldén, H. W. 1961. On the variation, nomenclature, distribution, and taxonomic position of *Limax (Lehmannia) valentianus* Férrusac (Gastropoda, Pulmonata). *Arkiv för Zoologi*, Series 2, 15: 71-96.

Journal of Alabama Academy of Science Vol. 77, No. 3-4, July/October 2006.

COMPARISON OF SELECTED SOILS PROPERTIES AT LANDSCAPE POSITIONS UNDER TROPICAL FOREST ECOSYSTEMS OF PUERTO RICO

Teferi D. Tsegaye[1], Reizelie Baretto[2], K. R. Islam[3], O. S. Mbuya[4] and Wagaw F. Mezemir[5]

[1, 5]Department of Plant and Science, Alabama A&M University, Normal, AL 35862, [2] The Pennsylvania State University, University Park, PA 16802, [3]Soil and Water Resources, The Ohio State University South Centers, Piketon, OH 45661, and center for Water Quality, Florida A&M University, Tallahassee, FL 32307-4100

Correspondence: Teferi Tsegaye: teferi.tsegaye@email.aamu.edu

ABSTRACT

Temporal changes in land-use exhibit considerable influence on soil properties. Our objective was to evaluate the impact of land-use practices within landscape positions on selected soil properties at Ciales, Guanica and Luquillo forest ecozones of Puerto Rico. At each landscape positions, ridges, slopes, and foothills were delineated to measure soil moisture content using Time Domain Reflectometry. Soil core samples were collected from 0-5, 5-10 and 10-15 cm depths, air-dried, processed, and analyzed for pH and exchangeable Ca, Mg, Al, and Fe contents. On average, pH decreased with increasing depth. The effect of landscape position on soil moisture content was more pronounced at Ciales than at Guanica and Luquillo. Soils at the Luquillo site contained significantly greater amounts of acidic cations, soils at the Ciales and Guanica sites contained greater amounts of basic and total cations. Greater amounts of Al and Mg were measured in soils from foothill soils than those obtained from ridge and slope at the Guanica site. Soils on ridges and slopes had Al saturation $\geq$70% compared to 50% in foothill soils. The amount of Al, Fe and acidic cations was greater in ridge soils at both Ciales and Luquillo, and in foothill soils at Guanica.

INTRODUCTION

Landscapes worldwide have been altered greatly in the last century by deforestation, fallowing, mining and agricultural operations. On the Caribbean island of Puerto Rico, most of the deforestation during the 1950's was the result of agricultural development activities such as growing coffee and sugar cane, and raising cattle (Dietz 1986). After World War II, the economic base of Puerto Rico shifted from agriculture to small industries (Dietz 1986). In the last 50 years, an industry-based economy resulted in partial

abandonment of agricultural activities throughout the island followed by an increase in secondary forest cover (15 to 30%) (Birdsey and Weaver 1987; Thomlinson et al., 1996).

Tropical forest ecosystems exhibit considerable temporal and spatial variability in response to land-use changes, and consequently can be extremely productive or extremely infertile (Sollins 1998). Since the impact of land-use and landscape position on functional components of terrestrial ecosystems often varies with time. Such factors, especially land-use practices, affect the degree of evapotranspiration and water infiltration, thereby modifying the soil moisture content and, consequently, net primary production, litter decomposition and release of nutrients (Birkeland 1984, Lal 1996). Changes in land-use practices lead to modifications of the soil properties, and can induce changes in the stability of the soil to carry out ecological functions.

Research on the impacts of land-use changes on soil is important to determine how fertility can be maintained and land-use systems improved. If we understand soil processes under secondary forest succession in abandoned pastures or fields, we may be able to accelerate the transition to forest or other systems. A natural establishment of forest and associated vegetation on abandoned lands improves the stability of the terrestrial ecosystems by promoting recovery of soil's functional properties (Islam and Weil 2000, Islam et al. 2001). A number of studies on impacts of land-use systems have showed important biological, chemical and physical modifications of the soils in tropical forest ecosystems (Lal 1996, Islam and Weil 2000, Islam et al. 2001).

An understanding of the impact of land-use practices and landscape position on soil properties in tropical rain forest ecosystems has environmental and economic importance. However, only limited work has been done to distinguish the effects of site, landscape position, and vegetation type on soil properties in the tropical forest ecosystems of Puerto Rico. In the present study, we assess the impacts of deforestation and conversion to pasture and agriculture, and the regeneration of secondary forest on selected moisture content and pH, exchangeable aluminum, iron, calcium, magnesium contents, and base saturation of soils on ridges, slopes and foothills in different ecozones of Puerto Rico.

MATERIALS AND METHODS

Description of the sampling sites:

The study was conducted at three different ecozones in the tropical forest ecosystems of Puerto Rico. The sampling locations were Ciales, Guanica, and Luquillo (Sabana) (Fig. 1). The Sabana research site is located in the northeastern part of the Luquillo Mountains of Puerto Rico. The existing native vegetation at this site is Tabonuco forest (subtropical wet forest), which is found below 600 m. (Ewel and Whitmore 1973). It is dominated by *Dacryodes excelsa*, *Manilkara bidentata* and *Sloanea berteriana*, and occupies nearly 70% of the Luquillo Experiment Forest (Ewel and Whitmore 1973, Cox et al. 2002). There is a small seasonal variation in rainfall (annual rainfall approximates 3500-mm) and air temperature (22 to 26^{0}C) (Brown et al., 1983; Scatena 1989). Soils at

Figure 1. Map showing sampling sites in Puerto Rico (Source: USDA-NRCS).

each of the landscape positions were created from volcanoclastic marine deposits (e.g. sandstones) during the Cretaceous era and subsequently uplifted during the Tertiary times (Scatena 1989). All the soils are complexes of Zarzal clays, an oxisol, and either Cristal or Humatas clays, which are ultisols (Johnston 1992). Three landscape positions (e.g. ridge, slope and foothill) adjacent to each other with similar slope characteristics were randomly selected from aerial photographs acquired in 1936, 1951, 1964, 1971, 1977, 1983, 1988, 1991, and 1993 (Zou and Gonzalez 1997). Ridges are local divides that receive no upland runoff, have gentle convex slopes, and divergent flow lines for runoff. Slopes receive runoff from upland areas and transmit runoff to valleys. Valleys are areas where flow lines converge and runoff concentrates (Scatena and Lugo 1995). These positions have similar soils and microclimates, but they differ in temporal land-use practices. The first landscape position, selected at the ridge top was deforested and converted to pasture prior to 1936. It was abandoned from pasture use in 1971 allowing to regenerate with native vegetation; it is now covered by secondary forest with an age range from 26 to 33-y (Aide et al. 1995). The second landscape position, selected was at middle slope, which was deforested and converted to pasture prior to 1936. However, this position was maintained as an active pasture until six months prior to our sampling. The third landscape position, selected at the foothill has been under mature forest cover since 1936.

The Guanica research site, located in the southwest part of Puerto Rico, has been dominated by subtropical dry forest since 1919. This site is part of the United Nations Biosphere Reserve, which covers more than 4000-ha with 57 km of trails and old roads. This subtropical dry forest supports a diverse variety of animal and plant spp. This area was used as a plantation for Campeche, mesquite and zarcilla, besides being used as cow pasture. This region receives an average of 750-mm rainfall annually, with a pronounced dry season from December through July. This extended dry season is occasionally interrupted by heavy rainfall during the month of May. Average annual temperature is 25^{0}C. Three sampling positions, viz. the Fuerte road at ridge top, Gutierrez on the slope, and the La Entrada at the foothill were selected. The forest at the Fuerte road site had been subjected to selective felling about 60-years ago, and the harvested wood was buried in pits for

charcoal production. The presence of residual charcoal was evident during our sampling. The forests at the Gutierrez and La Entrada sites were clear-felled for agriculture more than 40-years ago. In the past 30–40 years, the agricultural practices at these sites were abandoned and secondary forests were allowed to regenerate naturally. The Gutierrez site is currently being used for agriculture and secondary forest cover. On the other hand, the La Entrada site is strictly under secondary forest cover.

The Ciales research site is located in the Karst region (Lares Limestone formation) of Puerto Rico and is dominated by a subtropical moist forest (Ewel and Whitmore 1973). The limestone karst region extends along the north coast of Puerto Rico between the towns of Aguada and Loiza. The area is underlain by six limestone formations that range in age from Oligocene to Miocene (Giusty 1978). It is the second oldest limestone formation in Puerto Rico (Monroe 1976, Giusty 1978). The average annual rainfall in the town of Ciales is about 2100-mm. The vegetation composition at this site depends on the landscape position and orientation. Aerial photographs acquired from 1936 to 1971 showed that all of the valleys were used either for pasture, production of coffee and crops. Houses were located on slopes and dry valleys between mogote tops. The hilltops had dry evergreen forest, and the slopes, valleys and sinkholes had evergreen and seasonal forests. The foothills were covered with lower montane rain forest (Murphy 1916, Chinea 1980).

Soil sampling, processing and analysis:

Within each site, on-site moisture content was determined and soil samples at ridge, slope and foothill landscape positions were collected. On-site volumetric soil moisture content was determined by inserting a TRIME-FM three 5-cm rods Time Domain Reflectometry (TDR) probe into the top 5-cm soil depth. After determining on-site moisture content, a total of 193 composite soil samples were collected at 0-15-cm depth (segmented at 5-cm) from the above-mentioned sampling sites and landscape positions. Out of 193 soil samples, 81 samples were collected from both Guanica and Luquillo; however, in Ciales, only 31 samples were collected due to extreme dryness of the site. The collected soil samples were air-dried at room temperature for 3-d, ground using a mortar and pestle, and passed through a 2-mm sieve prior to analysis.

Soil pH was determined by using a glass electrode pH meter with a 1:1 ratio of 0.01M $CaCl_2$ solution: to soil. The Mehlich-1 solution was used to extract acidic and basic cations, such as aluminum, iron, calcium and magnesium. Exactly 5-g of soil was taken in 50-mL volumetric flasks followed by addition of 20-mL of 0.01-M Mehlic-1 solution. After 5-min of shaking, the soil suspensions were filtered to obtain clear aliquots. The concentration of metals such as Al, Fe, Ca, and Mg was measured by using an Inductively Coupled Plasma (ICP) spectrophotometer. The Aluminum, Iron, Calcium and Magnesium content for the 0-5, 5-10 and 10-15 cm depth increments were combined to calculate the amount of Aluminum, Iron, Calcium and Magnesium contents, and acidic, basic and total cations in the 0-15 cm soil.

Statistical analysis:

On-site volumetric soil moisture content and chemical analysis data were arranged

in a factorial form (3 sites x 3 positions x 3 depths) to analyze by using the GLM procedure of SAS (SAS Institute 2001). Using F-protected LSD at $p < 0.05$ we separated significant differences in treatment means and interactions.

RESULTS

Differences among sites, landscape positions, and vegetation types reflected soil properties (e.g. soil moisture) that are typically related to inputs of water (Fig. 2). In each site, differences among landscape positions reflected soil chemical reaction and elements (e.g. pH, Al, Fe, Ca, and Mg) that typically are associated with geochemical processes (Table 1-8 and Fig. 3).

Volumetric moisture content of soil varied significantly among sampling sites and among landscape positions under different land-use/land covers (Figure 2). On average, the Ciales site had significantly greater (>65%) soil moisture contents than the Guanica site. Volumetric moisture content of soil did not differ significantly between the Ciales and Luquillo sites. Averaged across sampling sites, foothill soils at both Ciales and Luquillo had greater moisture contents (>18-27%) than soils at slopes and ridges. Soil moisture content did not differ significantly between slopes and ridges. The effect of landscape position on volumetric moisture content was more pronounced in soils at Ciales than in soils at Guanica and Luquillo sites.

Averaged across landscape positions and soil depths, pH varied significantly among these sites (Table 1). Mean soil pH was higher for the Guanica and Ciales sites compared to the Luquillo site, which is highly acidic in nature (Table 1). In Ciales, landscape position had a significant effect on soil pH (Table 2). Soils on the ridges were more acidic than soils on slopes and foothills. Soils at different landscape positions of the Guanica and Ciales sites were slightly acidic to neutral compared to strongly acidic soils at the Luquillo site (Table 4). On average, pH gradually decreased with increasing soil depth.

The amount of exchangeable cations (e.g. Al, Fe, Ca and Mg) varied significantly among sites, landscape positions, and soil depth (Table 4). Soils collected from the Luquillo had a significantly greater content of exchangeable Al and Fe with correspondingly lower amounts of exchangeable Ca and Mg than in soils at Ciales and Guanica. However, the Al, Fe and Ca content did not differ significantly between the Ciales and Guanica sites. The Mg content also varied significantly among sites (Table 1). Soils on ridges at Ciales had significantly greater content of Al than did soils on slopes and foothills (Table 2). On the other hand, soils on slopes contained greater amounts of Ca and Mg than did soils on both ridges and foothills at the Ciales. Greater amounts of Al and Mg were measured in foothill soils than in ridge and slope soils at the Guanica (Table 3). In Luquillo, Fe content increased whereas Al, Ca and Mg content decreased with increasing soil depth (Table 4).

There were significant differences in acidic (Al + Fe), basic (Ca + Mg), total cations, and base saturation among sites, landscape positions and soil depths (Table 1 - 4). While soils at the Luquillo contained significantly greater amount of acidic cations, the soils at the Ciales and Guanica contained greater amounts of basic and total cations (Table 1 - 4). Soils at Guanica contained significantly greater concentration levels of basic and total cations than soils at Ciales. Soils on ridges at Ciales and Luquillo contained greater amounts of acidic cations than did soils on slopes and foothills (Table 5). Greater quantities

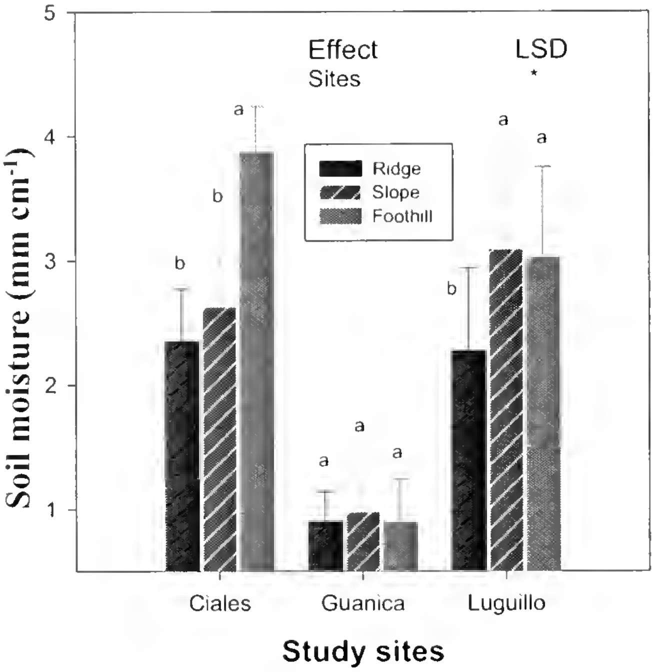


Figure 2. Soil moisture content at different landscape positions of sites in Puerto Rico.

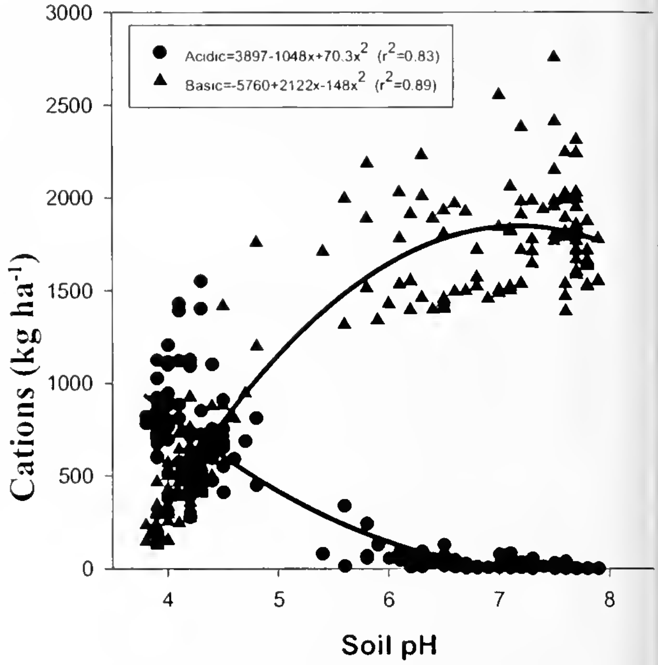


Figure 3. Relationship between pH and acidic and basic cation contents at different depths of soil.

of basic and total cations were also measured in foothill soils compared with soils on slopes and ridges at the Luquillo (Table 7). On average, surface soils (0-5-cm depth) contained greater amounts of basic cations than did subsurface soils (5-15-cm) at all three sites, and the distribution was more pronounced at the Luquillo site (Table 5 - 7).

Base saturation of soils at Luquillo was <40% while the Al saturation was >60% compared to soils at the Ciales and Guanica (Table 1). Soils on ridges and slopes had Al saturation ≥70% compared to 50% in foothill soils. At both Ciales and Luquillo, the foothill soils had greater base saturation than in soils on ridges and slopes (Table 5 - 7). Base saturation decreased with an associated increased in Al saturation in response to soil depth (Table 7). Base saturation did not vary in response to landscape positions or soil depth at Guanica (Table 6).

Compared over soil depth (0-15-cm), the amount of exchangeable Al, Fe and acidic cations was significantly greater at Luquillo than at Ciales and Guanica sites (Table 8). The amount of Ca in soil was greater at Ciales and Guanica than at Luquillo whereas the Guanica site had greater amounts of Mg and basic and total cations than the Ciales and Luquillo sites. The amount of Al, Fe and acidic cations were greater in ridge soils at both Ciales and Luquillo, and in foothill soils at Guanica. The foothill soils at Luquillo contained greater amounts of Ca, Mg, and basic and total cations than did ridge and slope soils. Soils on both ridges and foothills at Guanica contained greater amounts of Mg and basic and total cations than in soils on slopes. In Ciales, Mg and basic cations were greater in soils on slopes than in soils on ridges and foothills.

DISCUSSION

Significantly greater moisture content of soil at both Ciales and Luquillo is perhaps related to higher annual rainfall and increased forest cover. It is reported that tropical forest cover often improved soil condition by facilitating greater infiltration of water from rainfall with an associated decrease in surface runoff and evaporation (Islam et al. 2001). Greater moisture holding capacity of foothill soils as compared to soils on slopes and ridges at both Ciales and Luquillo may be related to deposition of finer soil particles (e.g. silt and clays) by surface runoff in response to previous land-use practices between 1936 and 1998 in most parts of Puerto Rico, especially in Luquillo (Thomlinson et al. 1996). Although by 1988 most of the agricultural lands were replaced by secondary forest cover, prior to 1988, cultivated sugar cane and pasture were dominant land-use practices in Luquillo. Temporal deposition of finer soil particles at the valley and foothill positions in response to accelerated surface runoff from soils on ridges and slopes, under extensive sugar cane cultivation, has significantly increased the moisture content throughout the soil profile. In addition, higher moisture content in the foothill positions of the landscapes at Ciales and Luquillo which is due to seepage from the upper slopes. Greater moisture content of soils on slopes at Luquillo is perhaps related to higher infiltration of water from rainfall under dense vegetation. The comparatively lower moisture content at the Guanica is possibly due to the combined effect of compaction and hard setting characteristics of soils, reduced rainfall infiltration due to the loss of transmission pores and surface crusting. Also there is less annual rainfall received, and more exposure to direct sunlight with a prolonged dry season from December to July.

Prevalence of highly acidic soils with very low amounts of exchangeable Ca and Mg or base saturation at the Luquillo site is perhaps related to weathering of acidic parent materials (Scatena 1989, Johnston 1992). Since rainfall events over a large portion of the tropical forest in the Luquillo region exceeded evapotranspiration for much of the year, there is a greater possibility of surface runoff, seepage and leaching over time. When rainfall percolates through the soil, as in the case at Luquillo site, it is most likely leaches the basic cations such as Ca and Mg, and replaces the exchange sites with acid-forming cations such as hydrogen, Al and Fe (Kamprath 1970). Significantly lower values of Ca and Mg suggest that the soils are impoverished with respect to temporal leaching of basic cations. Specifically, it has been proposed that Al mobilization in mineral acidic soil reduces storage of Ca (Shortle and Smith 1988, Bondietti et al. 1990, Lawrence et al. 1995). The dominance of low activity components (Al and Fe) in the soils of the humid forest zone also reflects the high degree of weathering (Nakna and Tonye 2003). Due to the low value of soil pH (< 5.5), Al could be present in exchangeable form (Nakna and Tonye 2003). Sanchez et al. (1982) reported that soils with 10-60% Al saturation present acidity problems while soils above 60% saturation exhibit Al toxicity. Therefore, acidity problems may occur in the topsoil and Al toxicity problems in the subsoil at the Luquillo site.

Table 1. Land-use effects on pH, and extractable calcium, magnesium, iron and aluminum contents, acidic (Al + Fe), basic (Ca + Mg) and total cations, and base saturation of soil at Ciales, Guanica, and Luquillo, Puerto Rico

Sampling Site	pH (1:1)	Al	Fe	Acidic cations	Ca	Mg	Basic cations	Total cations	Base saturation	Al saturation
		(kg ha^{-1})							(%)	
Ciales	6.7b	21.8b	0.32b	22.2b	853.3a	95.8c	946.7b	971.3b	97.5a	2.5b
Guanica	7.3a	11.4b	0.34b	11.9b	859.6a	326.5a	1187.3a	1198.7a	90.4a	9.6b
Luquillo	4.1c	427.5a	59.1a	486.6a	166.4b	137.9b	295.8c	787.3c	35.0b	65.0a

Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by same letters within a column are not significantly different at $p<0.05$.

Table 2. Landscape position and land-use effects on pH, and extractable calcium, magnesium, iron and aluminum content at different depths of soil in Ciales, Puerto Rico

Landscape Position	Land-use practices	Depth of soil (cm)	pH (1:1)	Al	Fe (kg ha^{-1})	Ca	Mg
Ridge	DRF	0-5	6.2a	16.4c	0.42a	834.4a	130.1a
		5-10	6.0ab	64.2b	0.44a	842.0a	75.9b
		10-15	5.9b	101.9a	0.47a	800.0a	44.7c
			6.0C	66.4A	0.45A	824.5B	77.7B
Slope	EGF	0-5	7.5a	0.25a	0.35a	857.8a	135.9a
		5-10	7.5a	0.56a	0.37a	891.6a	104.6a
		10-15	7.4a	0.72a	0.21a	871.3a	139.2a
			7.5A	0.51B	0.29A	873.6A	126.6A
Foothill	MRF	0-5	7.0a	2.1b	0.36a	892.4a	102.0a
		5-10	6.7b	6.5b	0.33a	847.5a	77.0b
		10-15	6.7b	11.6a	0.24a	844.3a	66.0b
			6.8B	6.8B	0.31A	861.4AB	81.7B

DRF=Dry forest, EGF=Evergreen forest, MRF=Montane rain forest, Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

It is reported that soils at the Guanica and Ciales are derived from uplifted marine limestone deposits and the Lares limestone formation, and contain a higher percent of carbonate minerals (Ewel and Whitmore 1973, Giusty 1978). The abundance of such alkaline minerals at the Guanica and Ciales sites is possibly due to the greater amount of Ca and Mg to maintain neutral pH and high base saturation in the soil. The relatively high base saturation and the low Al saturation in the soil reflected the saturation status of the exchange complex with basic cations. A smaller amount of exchangeable Al in the Ciales and Guanica soils could be due to complexation with organic matter. A significant positive correlation of soil pH with basic cations (e.g. Ca and Mg) and a negative correlation with acidic cations (e.g. Fe and Al) supported our results (Fig. 3). Such natural processes as well as land-use changes are possibly responsible for soils being highly acidic with greater Al saturation at Luquillo, and slightly acidic to neutral with greater base saturation at Ciales and Guanica.

Table 3. Landscape position and land-use effects on pH, and extractable calcium, magnesium, iron and aluminum content at different depths of soil in Guanica, Puerto Rico

Landscape Position	Land-use practices	Depth of soil (cm)	pH (1:1)	Al	Fe (kg ha^{-1})	Ca	Mg
Ridge	SCF	0-5	7.5a	2.0a	0.15a	868.5a	456.7a
		5-10	7.7a	0.27a	0.10a	847.3a	298.6b
		10-15	7.7a	0.17a	0.07a	879.1a	258.7b
			7.6A	0.91B	0.11A	864.5A	347.4A
Slope	ASF	0-5	7.5a	5.6a	0.25a	856.1a	311.4a
		5-10	7.6a	7.5a	0.13a	892.2a	237.9b
		10-15	7.7a	9.3a	0.17a	885.3a	214.0b
			7.6A	7.4B	0.18A	877.6A	256.0B
Foothill	SFR	0-5	6.4b	22.5a	0.62a	823.0a	377.2a
		5-10	6.9a	25.7a	0.76a	862.2a	399.7a
		10-15	7.0a	26.4a	0.61a	830.4a	357.1a
			6.8B	24.9A	0.66A	838.6B	378.0A

SCF=Selective cut forest, ASF=Agriculture and secondary forest, SFR=Secondary forest, Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

Although there were significant differences in soils among sites, a variation in Al, Fe, Ca and Mg content of adjacent soils in different landscape positions within the Luquillo site may be influenced by the gravitational transfer of water, mass and nutrients. The processes and/or properties responsible for such differences are perhaps that the lateral flow of nutrient-rich water that removes cations from soils on ridges and slopes and deposits them in foothills and valleys, and an accumulation of Al and Fe oxides in soils on ridges and slopes (Cox et al. 2002). Likewise, in Luquillo soils, where lateral through flow occurs, basic cations in solution or suspension are transported down slope and accumulate in foothills and valleys; this would account for the higher concentrations of Ca and Mg in the soils on foothills and valleys.

Table 4. Landscape position and land-use effects on pH, and extractable calcium, magnesium, iron and aluminum content at different depths of soil in Luquillo, Puerto Rico

Landscape Position	Land-use practices	Depth of soil (cm)	pH (1:1)	Al	Fe (kg ha^{-1})	Ca	Mg
Ridge	SFR	0-5	4.1a	641.2a	64.7b	171.2a	164.4a
		5-10	4.0a	547.5b	72.8ab	91.2b	115.5b
		10-15	3.9a	421.8c	85.4a	51.0c	96.7b
			4.0A	536.8A	74.3A	104.5B	125.5B
Slope	Pasture	0-5	4.0a	356.2a	65.7b	141.3a	127.8a
		5-10	4.0a	374.2a	82.7a	97.6b	84.7b
		10-15	3.9a	348.3a	81.4a	95.4b	77.5b
			4.0A	359.5B	76.2A	112.6B	97.9C
Foothill	MFR	0-5	4.5a	389.6a	27.0a	340.5c	232.0a
		10-10	4.4a	419.0a	34.6a	278.1b	174.2b
		10-15	4.3a	381.8a	33.9a	175.0c	140.9b
			4.4B	396.0B	31.7B	264.1A	182.6A

SFR=Secondary forest, MRF=Mature forest, Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

Table 5. Landscape position and land-use effects on acidic (Al + Fe), basic (Ca + Mg), and total cation content, and base saturation at different depths of soil in Ciales, Puerto Rico

Landscape Position	Land-use practices	Depth of soil (cm)	Acidic cations	Basic cations	Total cations	Base saturation	Al saturation
			(kg ha^{-1})			(%)	
Ridge	DRF	0-5	16.8c	964.4a	981.2a	98.3a	1.7c
		5-10	64.6b	917.9ab	982.5a	93.4ab	6.6b
		10-15	102.4a	845.1b	947.5a	89.2b	10.8a
			66.8A	902.2B	969.0A	93.6B	6.4A
Slope	EGF	0-5	0.60a	993.7a	994.3a	99.9a	0.1a
		5-10	0.46a	996.2a	996.7a	99.9a	0.1a
		10-15	0.93a	1010.5a	1011.4a	99.9a	0.1a
			0.66B	1000.1A	1000.8A	99.9A	0.1B
Foothill	MRF	0-5	2.5b	994.4a	996.9a	99.7a	0.3a
		5-10	6.9ab	924.5a	931.4a	99.3a	0.7a
		10-15	11.8a	910.2a	922.1a	98.7a	1.3a
			7.1B	943.0AB	950.1A	99.1A	0.9B

DRF=Dry forest, EGF=Evergreen forest, and MRF=Montane rain forest. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

Table 6. Landscape position and land-use effects on acidic (Al + Fe), basic (Ca + Mg), and total cation content, and base saturation at different depths of soil in Guanica, Puerto Rico

Landscape Position	Land-use practices	Depth of soil (cm)	Acidic cations	Basic cations (kg ha^{-1})	Total cations	Base saturation (%)	Al saturation
Ridge	SCF	0-5	2.2a	1325.2a	1327.2a	99.8a	0.2a
		5-10	0.37a	1145.9b	1146.2b	99.9a	0.1a
		10-15	0.22a	1137.8b	1138.0b	99.9a	0.1a
			1.0C	1211.9A	1212.9A	99.9A	0.1A
Slope	ASF	0-5	5.9a	1167.6a	1173.5a	99.5a	0.5a
		5-10	7.6a	1130.1a	1137.7a	99.4a	0.6a
		10-15	9.4a	1099.4a	1108.9a	99.1a	0.9a
			7.6B	1133.6A	1223.3A	99.3A	0.7A
Foothill	SFR	0-5	23.1a	1200.2a	1223.3a	98.1a	1.9a
		5-10	26.5a	1261.9a	1288.4a	98.0a	0.2a
		10-15	27.0a	1187.5a	1214.5a	97.8a	2.2a
			25.5A	1216.6A	1242.1A	97.9A	2.1A

SCF=Selective cut forest, ASF=Agriculture and secondary forest, SFR=Secondary forest, Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

Table 7. Landscape position and land-use effects on acidic (Al + Fe), basic (Ca + Mg), and total cation content, and base saturation at different depths of soil in Luquillo, Puerto Rico

Landscape Position	Land-use practices	Depth of soil (cm)	Acidic cations	Basic cations	Total cations	Base saturation	Al saturation
			(kg ha^{-1})			(%)	
Ridge	SFR	0-5	705.9a	335.6a	1041.4a	32.5a	61.5b
		5-10	620.4b	206.7b	827.0b	25.1ab	74.9ab
		10-15	507.2c	147.8c	655.0c	22.3b	77.7a
			611.2A	230.0B	841.2A	26.7B	73.3A
Slope	Pasture	0-5	421.9a	269.1a	691.0a	39.8a	61.2b
		5-10	456.9a	182.3b	639.3a	24.6b	75.4a
		10-15	429.6a	173.0b	602.6a	25.7b	74.3a
			435.6B	210.6B	646.2B	30.0B	70.0A
Foothill	MRF	0-5	416.6a	572.4a	989.0a	57.3a	43.7b
		5-10	453.6a	452.2b	905.8a	44.4ab	55.6ab
		10-15	415.7a	316.0c	731.6b	42.8b	57.2a
			427.8B	446.7A	874.5A	48.2A	52.8B

SFR=Secondary forest, MRF=Montane rain forest, Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

Table 8. Land-use effects on pH, and Calcium, Magnesium, Iron and Aluminum content, acidic (aluminum and iron), basic (calcium and magnesium) and total cations, and base saturation of soil profile (0-15-cm) at different landscape position of Ciales, Guanica, and Luquillo sites in Puerto Rico

Sampling Site	Landscape position	Land-use practices	Al	Fe	Acidic cations	Ca	Mg	Basic cations	Total cations
			(kg ha^{-1})						
Ciales	Ridge	DRF	182.5a	1.3a	183.8a	2476.4a	250.7b	2727.1a	2910.9a
	Slope	EGF	1.5c	0.9a	2.4c	2620.8a	379.8a	3000.6a	3003.0a
	Foothill	MRF	20.2b	0.9a	21.1b	2584.2a	245.1b	2829.3a	2850.4a
			68.1B	1.0B	69.1B	2560.5A	291.9B	2852.3B	2921.4B
Guanica	Ridge	SCF	2.4c	0.34a	2.7c	2593.5a	1042.2a	3635.7a	3638.4a
	Slope	ASF	21.4b	0.52a	21.9b	2635.3a	739.6b	3374.9b	3396.8b
	Foothill	SEC	74.6a	1.98a	76.6a	2515.7a	1133.9a	3649.6a	3726.2a
			32.7B	0.95B	33.6B	2581.5A	971.9A	3550.7A	3585.0A
Luquillo	Ridge	SEF	1610.5a	222.9a	1833.4a	313.4b	376.5b	690.0b	2523.5a
	Slope	PAS	998.5c	211.5a	1210.0b	312.9b	272.0c	584.9b	1794.9b
	Foothill	MAF	1148.5b	92.0b	1240.5b	765.8a	529.6a	1295.4a	2535.9a
			1252.5A	175.5A	1428.0A	464.0B	392.7B	856.8C	2284.8C

DRF=Dry forest, SEF=Secondary forest, SCF=Selective cut forest, EGF=Evergreen forest, MRF=Montane rain forest, ASF=Agriculture and secondary forest, MAF=Mature forest, and PAS=Pastures, Al=Aluminum, Fe=Iron, Ca=Calcium, and Mg=Magnesium. Means separated by uppercase letters within a column are significantly different at $p<0.05$. Means separated by lowercase letters within a category of column are significantly different at $p<0.05$.

CONCLUSIONS

The rainfall distribution, land use practices and natural vegetation, and parent materials were dissimilar among the sites, accounting for most of the variations measured in soil properties. At Ciales and Luquillo, moisture content of soil was quite high, suggesting that greater annual rainfall and dense forest cover may be responsible for the infiltration and redistribution of moisture in the soil profile. Prevalence of highly acidic soil at the Luquillo site is related to parent material and accelerated leaching of Ca and Mg. Greater Al saturation causes acidity in topsoil and Al toxicity problems in the subsoil at Luquillo. A significant positive correlation of soil pH with basic cations (e.g. Ca and Mg) and a negative correlation with acidic cations (e.g. Fe and Al) corroborated our results. Since soils within each site developed from the similar parent materials, existing differences in exchangeable Al, Fe, Ca and Mg contents among landscape positions might have been influenced by vegetative cover and soil erosion. The processes and/or properties responsible for such differences are perhaps the lateral flow of nutrient-rich waters from soils on ridges and slopes followed by a deposition in foothills and valleys, and the accumulation of Al and Fe oxides in soils on ridges and slopes at Luquillo.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Center for Hydrology, Soil Climatology, and Remote Sensing (HSCaRS) support staff. Contribution is also acknowledged from the HSCaRS Center and Agricultural Experiment Station, Alabama A&M University, Normal, AL 35762, Journal No. 566. This work was supported by National Aeronautics and Space Administration (NASA), Grant No. NAGS-10721 Washington, DC. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

REFERENCES

Aide, T. M., J. K. Zimmerman, L. Herrera, M. Rosario, and M. Serrano. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. Forest Ecology and Management 77: 77-86.

Birdsey, R. A., and P. L.Weaver. 1987. Forest area trends in Puerto Rico. Res. Note, SO-331, US Forest Service, New Orleans, LA 13 pp.

Birkeland, P.W. 1984. Soils and Geomorphology. Oxford Univ. Press, New York.

Bondietti, E.A., N. Momoshima, and W.C. Shortle, and K.T. Smith (1990). A historical perspective on divalent cation trends in red spruce stem wood and the hypothetical relationship of acid deposition. Canadian Journal of Forest Resources 20: 1850-1858.

Bouwman, A.F. 1990. Exchange of greenhouse gasses between terrestrial ecosystems and the atmosphere. P. 61-127. In A.F. Bouwman (ed.) Soils and the greenhouse effect. John Wiley and Sons, Chichester, UK.

Chinea, J. D. 1980. The forest vegetation of the limestone hills of northern Puerto Rico. Cornell University, Master Thesis.

Cox, S.B., M.R. Willig, and F.N. Scatena (2002). Variation in nutrient characteristics of surface soils from the Luquillo Experimental Forest of Puerto Rico: A multivariate perspective. Plant and Soil 247: 189-198.

Dietz, J. L. (1986) Economic history of Puerto Rico: Institutional change and capitalist development. Princeton University Press, Princeton, NJ, 337 pp.

Ewel, J.J. and J. L. Whitmore. (1973) The ecological life zones of Puerto Rico and the U.S. Virgin Islands. USDA-Forest Ser. Res. Paper, ITF-18. Ins. Tropical Forestry, Rio Piedras, Puerto Rico.

Guisty, E. V. 1978. Hydrogeology of the karst of Puerto Rico. U.S. Geol. Surv. Prof. Paper.

Islam, K.R., and R.R. Weil (2000) Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. Agriculture, Ecosystems and Environment. 79:9-16.

Islam, K.R., M.R. Ahmed, M. K. Bhuiyan, and A. Badruddin. (2001) Deforestation effects on vegetative regeneration and soil quality in tropical semi-evergreen degraded and protected forests of Bangladesh. Land Degradation and Development 11:1-12.

Johnston, M.H. (1992). Soil-vegetation relationships in a Tabonuco forest community in the Luquillo Mountains of Puerto Rico. J. Tropical Ecology. 8: 253-263.

Kamprath, E.J. (1970). Exchangeable aluminum as a criterion for liming leached mineral soils. Soil Science Society of America Proceedings 24: 252-254.

Lal, R. (1996) Deforestation and land-use effects on soil degradation and rehabilitation in Western Nigeria. I. Soil physical and hydrological properties. Land Degradation and Development 7: 19-45.

Lawrence, G.B., M.B. David, and W.C. Shortle (1995). A new mechanism for calcium loss in forest-floor soils. Nature 378: 162-165.

Monoroe, W. H. 1976. The karst land forms of Puerto Rico. U.S. Geol. Surv. Prof. Paper 899.

Murphy, L. S. 1916. Forests of Puerto Rico, past, present, and future, and their physical and economic environment. USDA Bull. 354, Washington, DC, 99 pp.

Nakna, J.C.V., J. Tonye. (2003) Assessment of certain soil properties related to different land-use systems in the Kaya watershed of the humid forest zone of Cameroon. Land Degradation and Development 14: 57-67.

Sanchez, P.A., W. Couto, and S.W. Buol (1982). The fertility capability soil classification system: Interpretation, applicability and modification. Geoderma 27: 283-309.

SAS Institute. 2001. SAS Users' Guide. R 8.2; SAS Institute, Inc., Cary, NC.

Scatena, F.N. (1989). An introduction to the physiography and history of the Bisley Experimental Watersheds in the Luquillo Mountains of Puerto Rico. General Technical Report. SO-72. USDA Forest Service Southern Forest Expt. Station, Rio Piedras, Puerto Rico, pp. 22

Scatena, F.N., and A.E. Lugo (1995). Geomorphology, disturbance, and the soil and vegetation of two subtropical wet steep land watersheds of Puerto Rico. Geomorphology 13: 199-213.

Shortle, W.C., and K.T. Smith (1988). Aluminum-induced calcium deficiency syndrome in declining red spruce. Science 240: 1017-1018.

Sollins, P. (1998) Factors influencing species composition in tropical lowland rain forest: Does soil matter? Ecology 79: 23-30.

Thomlinson, J. R., M. I. Serrano, T. M. Lopez, T. M. Aide, and J. K. Zimmerman. 1996. Land use dynamics in post-agricultural Puerto Rican landscape (1936-1988). Biotropica, 28: 525-536.

Zou, X., and G. Gonzalez. 1997. Changes in earthworm density and community structure during secondary succession in abandoned tropical pastures. Soil Biol. Biochem. 29:627-629.

BOOK REVIEW

HUMAN TRANSFORMATION IN THE TWENTY-FIRST CENTURY?

James T. Bradley
Department of Biological Sciences
Auburn University
Auburn, AL 36849
bradljt@auburn.edu

Radical Evolution: The Promise and Peril of Enhancing Our Minds, Our Bodies - and What It Means to Be Human. By Joel Garreau. New York, NY, Doubleday, 2005. 273 pages plus Suggested Readings, Notes and Index.

Several prominent thinkers and writers, and a few scientists and engineers, believe that humankind is poised to use technology to transcend its human nature acquired over eons of evolution by natural selection. Joel Garreau's *Radical Evolution* describes views of these futurists and four technologies that drive their forecasts - genetic, robotic, information, and nano processes. Garreau collectively calls these the GRIN technologies. How might these transform humans?

From genetics may come enhancement of human germ lines, age retardation, cloning and organ regeneration; robotics and information technologies may yield machines more intelligent than humans and just as autonomous; and nanotechnology is already used to construct materials and particles with dimensions of a few billionths of a meter and properties astonishingly different from those we encounter in everyday objects.

Developments in any one of these fields advance the others. Acting synergistically, their power to transform humans may be unmatched by all but two former periods in human history – (1) the transition to bipedality and the beginning of brain expansion about five million years ago, and (2) the Axial Age, a period of spiritual enlightenment between about 800 and 200 B.C.E. to which major religious and philosophical traditions trace their roots to figures like Socrates, Isaiah, Zoroaster, the Buddha, and Confucius.

Joel Garreau, neither a scientist nor a technologist, writes about global change and values. A reporter, editor and correspondent for *The Washington Post,* he has been a senior fellow at the University of California at Berkeley and at George Mason University. Garreau heads The Garreau Group, a network of persons committed to "understanding who we are, how we got that way, and where we're headed, worldwide."

The notion that humanity is now entering a major transformative period is predicated upon the reality of The Curve, the exponential growth in the power and complexity of human technologies. Existence of The Curve for information technology was noted in 1965 by Gordon E. Moore, who observed that the complexity of "minimum cost semiconductor components" doubled about every year. Moore's observation was soon

formalized into Moore's Law: the power of information technology will double every 18 months into the indefinite future. Garreau reports that the power of information technology has now followed Moore's Law for nearly 30 doublings. The current Deep Blue IBM computer project aimed at producing a machine that handles a thousand trillion instructions per second may approach the processing power of the human brain. If genetics, robotics and nanotechnology are also driven by The Curve, we and our world may be about to change, radically and perhaps irreversibly.

Garreau writes as though he believes all four GRIN technologies obey The Curve, although objective data for the belief exists only for information technology. For the sake of dialogue, let us assume that advances in all GRIN technologies do obey The Curve and will continue to do so into the indefinite future. What would this mean for humankind? 'Transcendence' is Garreau's answer.

We are not given one concise definition of transcendence, but Garreau does write an entire chapter titled "Transcend" in which he ponders his and others' notions of human transcendence. During an interview with Nick Bostrom, cofounder of the World Transhumanist Association, Garreau reports having asked: "When we're talking about transhumanism, we're talking about transcending human nature...One notion of transcendence is that you touch the face of God. Another version of transcendence is that you become God. Does the word *transcendence* mean anything to you?" Bostrom's lengthy response is optimistic about humankind's using technology to attain "higher levels of moral excellence" (p. 242). But everybody who thinks about transcendence does not emphasize morality. For example, interviewee Jaron Lanier, computer scientist, artist, composer, and originator of the term 'virtual reality,' points out that humankind's moral evolution can lead to holy wars (p. 210). I must agree. Do not clashing religious doctrines contribute to destruction and death in the Middle East and elsewhere? For Lanier, 'transcendence' means heightened feelings of connection between individuals.

The nature of 'transcendence' will depend upon the character of that which is being transcended, i.e., human nature. Nowhere is there consensus on how to define or describe human nature. Garreau personally seems to like the view of German sociologist Justin Stagl, which he (Garreau) summarizes as follows: "Our human nature may be grounded in our animal nature, but our ability and eagerness to develop our 'better nature' are unique." Three possible paths for society after humans transcend their present nature are imagined by Garreau - the Heaven, Hell and Prevail Scenarios. These are developed mainly from information obtained by interviewing dozens of people writing and/or thinking about the GRIN technologies and the future of humankind.

Ray Kurzweil and Gregory Stock are named spokespersons for the Heaven Scenario. Kurzweil is a computer scientist, inventor, author (*The Age of Spiritual Machines, When Computers Exceed Human Intelligence*, Penquin Putnam, New York, NY, 1999), and entrepreneur. Stock is a developmental biologist by training. He now heads the Program on Medicine, Technology and Society at UCLA's School of Medicine and a new biotechnology company in New Jersey. He is author of several books including *Redesigning Humans: Our Inevitable Genetic Future* (Houghton Mifflin Co., New York, NY, 2002).

Kurzweil is assiduous in promoting human cognitive enhancement via information

technology. Current humanity, he believes, is a transient stage in the overall evolutionary history of intelligence in the universe. He predicts that within a few decades our "wet brains" of neurons, synapses, and other cells and cell products will be augmented, perhaps even replaced, by non-biological computational and analytical devices. Kurzweil cites present brain implants to treat deafness and Parkinson's disease as early precursors of microscopic robots distributed by the billions throughout the brain to enhance cognition and create whatever virtual reality one desires. Kurzweil, now in his late 50's, also envisions literal immortality for humans within his lifetime through the freeing of our intellect and consciousness from its mortal, biological constraints. He views the body as a transient housing for the essence of an individual, the mind. When the mind can be contained in/on long-lived, non-biological material, biological aging and senility will be irrelevant to its continued existence. "What we see in evolution," says Kurzweil, "is increasingly accelerating intelligence, beauty...exponentially greater love" (p. 93).

A 'singularity' is what Kurzweil and others believe GRIN technologies will ultimately produce for humanity. The term is borrowed from mathematics and physics where it refers to a function that approaches infinity or a point of infinite density and energy such as at the Big Bang, where known laws of physics break down. Garreau explains that The Singularity in human history denotes a time when technology is transforming humanity and society so rapidly that "our everyday world stops making sense...that's when The Curve goes almost straight up. The sheer magnitude of each doubling becomes unfathomable" (p. 72). Soon after The Singularity arrives, before 2030 according to some, robotic intelligence will equal or eclipse that of human intelligence, and meaningful communication between enhanced and non-enhanced humans may be impossible. Exactly how downloading human minds onto pieces of long-lived, non-biological material translates into increased beauty and love is not explained by Kurzweil or Garreau.

Gregory Stock also sees radical human enhancement as inevitable, but first through the genetic manipulation of our biology rather than from enhancements from computer/information technology. Life extension, disease prevention and cures, rejuvenation of aging tissues and organs, and cognitive enhancements, he believes, will emerge from advances in and/or expanded use of existing technologies like *in vitro* fertilization, embryo selection, cloning and genetic enhancement. In Garreau's Heaven Scenario, humans will ultimately use GRIN technologies to create heaven on earth - a world where disease and even death have lost their sting and where war, greed, poverty and suffering are relics of our pre-transcendence history.

Then there is the Hell Scenario. Garreau assigns the ideas of computer scientist Bill Joy and author-historian Francis Fukuyama to the Hell Scenario. Bill Joy is cofounder and former Chief Scientist of Sun Microsystems. He also co-chaired the presidential commission on the future of information technology research. Francis Fukuyama is widely published as an international political economist and social historian, Dean of Faculty at the Paul H. Nitze School of Advanced International Studies, Johns Hopkins University, and a member of the president's Council on Bioethics.

Joy, in his 2000 *Wired Magazine* article, "Why the Future Doesn't Need Us," warns about the risks of GRIN technologies. He foresees disastrous outcomes from constructing self-replicating, biological pathogens and non-biological products of nanotechnology that

out compete naturally evolved biological inhabitants of the biosphere. Fukuyama is mainly concerned that using modern biotechnologies like cloning, age retardation and genetic enhancement to alter human nature will endanger liberal democracy, a view advanced in his 2002 book *Our Posthuman Future - Consequences of the Biotechnology Revolution* (Farrar, Straus and Giroux, New York, NY). Both authors believe that further development of GRIN technologies must be restricted in order to avert ruinous results from their use.

Somewhere between Heaven and Hell is a Prevail Scenario not polarized toward either optimism or pessimism. The Prevail Scenario views human history as an odyssey in which crises are dealt with as they arrive -- sometimes successfully and sometimes unsuccessfully. It does not deny the that GRIN technologies may follow The Curve, but it presumes that good decisions by ordinary people will guide humankind through difficult and confusing times as they have during earlier periods. Garreau's favorite spokesperson for Prevail is Jaron Lanier, who believes that humans can and will creatively shape the impact of technology on human nature and society to their benefit. In the Prevail future, increased and strengthened empathetic connections occur between individuals. Lanier refers to this increase in interpersonal connections as the "connectivity ramp" to distinguish it from exponential change associated with The Curve. In the Prevail Scenario, human will, imagination and courage separate The Curve from its effects on society.

Even more difficult than imagining how GRIN technologies may affect medicine, human cognition and communication is predicting their effects on religious beliefs and practices. Whether religion overall would fare differently in the Heaven, Hell and Prevail Scenarios, and whether different religious traditions would be affected differently, are interesting questions addressed only cursorily by Garreau. What we do get, primarily in Chapter 7 "Transcend," is a rich potpourri of Garreau's and others' musings on human nature and how technology-driven transcendence of human nature might affect our spirituality. Here, the narrative is driven more by questions than by conclusions. For example, what if humans transform themselves into angels? Kurzweil predicts that by 2099 human beings will have become indistinguishable from the Christian portrayal of angels -- immortal minds in nonphysical bodies that can be projected at will as real or virtual matter (p. 104). In religious systems where immortality and perfection are characteristics of God that distinguish us from Her/Him, how would the notion of 'humans' approaching immortality and perfection here on earth, through their own cleverness, be received?

Another question is whether genetic, pharmaceutical or electronic manipulations will ever remove religion as an essential human need. Garreau reports that history of religion author Karen Armstrong believes that humans are hardwired for religion (p.259). She is not alone. That religion has biological roots is the thesis of at least two relatively recent books (*Why God Won't Go Away* by A. Newberg, E. D'Aquili, and V. Rause, Random House, Inc., New York, NY, 2001; *The God Gene* by D. Hamer, Doubleday, New York, NY, 2004). If the need for religion is based on "a universal search for meaning and values" or an inability to "endure emptiness and desolation" (Armstrong, cited by Garreau, p. 259), is this need something we ought to change about our human nature?

What about spirituality and Lanier's 'connectivity ramp'? Martin E.P. Seligman, a former president of the American Psychological Association, says that a meaningful life "consists in attachment to something bigger than you are" (p. 261). Will our being part of

a large, diverse, compassionate, intimately connected group of people, as envisioned by Lanier, provide meaning to life in ways currently met by religion? In my view, if this is to be, Lanier's 'connectivity ramp' must touch us in ways qualitatively different from the ways cell phones, iPods and BlackBerries do now. Lanier himself embraces a cautious agnosticism when it comes to spirituality: "For a lot of these questions, I think 'I don't know' is the most dignified and profound answer." He leaves open the possibility that "the world we manipulate here isn't all there is. The world accessible by technologies isn't all there is" (p. 199).

So what are we to make of Garreau's book, his selection of interviewees, their predictions, and the future of human nature? Garreau is a talented writer. He has created an engaging narrative from an extraordinarily diverse pool of views and information gained from interviews and writings of dozens of persons. The categories of Heaven, Hell and Prevail undoubtedly oversimplify a real future revolutionized by GRIN technologies, but they serve well for organizing his narration and helping readers unfamiliar with GRIN technologies to order their thinking about them. Still, as a scientist and as a biologist, I will warn non-science trained readers of *Radical Evolution* about two things – the unspectacular nature of 'normal science,' and the misunderstood gene.

'Normal science' is Thomas Kuhn's term for the process of scientific discovery occurring between scientific revolutions (*The Structure of Scientific Revolutions*, 1962, University of Chicago Press, Chicago, IL). Normal science is performed working within a paradigm for the discipline – a set of premises about the way nature behaves. Periodically, observations that do not fit neatly into the current paradigm induce a crisis for the discipline that usually can be resolved by further or more careful experimentation, but which occasionally forces a paradigm shift, i.e. a scientific revolution. The 20th century transition in physics from a Newtonian viewpoint to a relativistic one is a classic example of a Kuhnian paradigm shift. Only a handful of scientists throughout history have been directly engaged in the 'extraordinary science' that causes a paradigm shift – e.g. Copernicus, Galileo, Einstein, Bohr, Heisenberg, Watson and Crick. By contrast, in every generation hundreds of thousands of researchers at universities and in industry throughout the world spend their professional careers doing 'normal science' – toiling away at their laboratory benches every day to provide, bit-by-painstaking-bit, basic information that ultimately becomes interpreted for general audiences by journalists and other writers like Garreau and most of his many sources.

With a few exceptions like Gregory Stock, whose professional career has included top-notch academic research and discovery, Garreau's sources do not include scientists working in the trenches of normal science. Trench-workers, largely unknown by journalists and the general public, are named as authors in the tables of contents of scientific periodicals like *Science*, *Nature*, *Cell*, *Proceedings of the National Academy of Sciences of the United States of America*, *Chemical Engineering Science*, *Nanotechnology*, *Journal of Physical Chemistry* and *Industrial and Engineering Chemistry Research*. These are the persons with intimate knowledge about the expense, time, effort, careers and collaborations it usually takes to obtain even modestly significant discoveries, not to mention the time and complexities involved in performing clinical trials and gaining federal approval before new materials or practices are offered to the public. In my view, these are also the persons to

interview for realistic insights into feasibility and timeframe estimations for realizing fruits of GRIN technologies.

Had *Radical Evolution* included the views of trench-workers on subjects like human limb regeneration, organ farming, tissue repair and rejuvenation by micro-robots, human minds freed from biological constraints, strong artificial intelligence and virtual human immortality, would readers finish the book with a different impression about humankind's future in this century than they do now? I suspect so. Most readers' views about the future, I believe, would be less radical. Transcendence of our present nature, whatever that nature is, will evolve gradually as an accumulation of nearly imperceptible changes rather than pouncing upon us as a shocking revolution. At least that is what I foresee for genetic and cellular-based technologies, those upon which I am most qualified to comment.

In addition to the laborious nature of normal science, widespread misconceptions about gene function are also bases for tempering expectations for transformative, biologically-based changes to human nature. French molecular biologist and historian of science, Michael Morange, wrote about the misunderstood gene in 1998, and an English translation of his book became available in 2001 (*The Misunderstood Gene*, Harvard University Press, Cambridge, MA, Transl. by Matthew Cobb). According to Morange, weaknesses in metaphors that have long been used to describe genes have engendered misunderstandings in the public and within a generation of scientists that has not experienced the historicity of those metaphors.

Until completion of the Human Genome Project (HGP) in 2001, it may not have mattered much whether one thought of an organism's genes as a book, blueprint or computer program. But now metaphors matter more because they powerfully influence payoff expectations from genomics. Metaphor-driven expectations usually overlook realities of the complexities of gene function and expression. For example, the 'book' and 'blueprint' metaphors falsely elevate the importance of genes above other cellular components. The 'computer program' metaphor does the same by implying that the program (DNA) commands the behavior of subservient proteins and that progressive development of an organism can be understood by decoding its genes. Views like this ignore the critical and complex interplay between proteins, cells, and their environment during development. Corollaries to metaphors placing genes at the apex of a regulatory pyramid of determinative events and elements include the belief that genomic information will be rapidly translated into cures for multitudinous genetic diseases and other human biological enhancements.

Gene knockout experiments in mice reveal our ignorance about gene function. Inactivating genes with known, vital functions often produces no observable defects in the mouse. This surprising result probably arises from one or more phenomena including functional gene redundancy and gene compensation; that is, other unidentified genes taking over the function of the knockout gene. In other experiments, knocking out a single gene with a single known function often produces unexpected structural or physiological changes seemingly unrelated to the known function of the gene. Possible explanations include the existence of complex networks of interdependent gene products and/or hierarchical structures in which the activity of a gene product depends on the action of many other gene products that create ordered, organic environments radiating outward with increasing

complexity (Morange, 2001). The upshot is that, with a few exceptions, adding, altering or deleting one or a few genes is not a safe or realistic approach to curing disease or enhancing specific biological attributes in the foreseeable future.

Working to increase our presently limited knowledge about the function of specific gene products and their interactions with other gene products is the field of proteomics, a relatively new subdiscipline of molecular biology. Proteomics examines the structure, occurrence and function of networks of protein-protein interactions. Already, its power has been demonstrated, such as by the recent report identifying genes for fertility factors that may someday be targets for male contraception (Chu, et al., 2006. *Nature* 443: 101-105).

Proteomics may someday make possible an era of prudent, positive eugenics through human genetic engineering. Although I doubt that this will happen very soon, I agree with Gregory Stock and paleontologist/evolutionary biologist George Gaylord Simpson that ultimately, human genetic enhancement is inevitable. Simpson's words reflect the care and humility with which the project must be undertaken: "I am pretty sure that if we survive, if we do not destroy ourselves with pollution, atomic war, and so on, we will sooner or later wish to take control of our own evolution. But I hope we do not do it too soon. I mean, at the moment we are far too ignorant, both of genetics and of what we really want, to tinker with our own evolution. So I'm not urging eugenic measures upon us. I'm really not. I hope we will not do that. But ultimately I'm sure we will. What we shall decide we want, of course, is up to our great great great grandchildren. But they will take this on." (From interview for video: *Origin of Species: Beyond Genesis*, Great Books Series. Walter Cronkite, ed.; Donald Sutherland, narrator, 1993).

What *Radical Evolution* provides is a heads-up on possible dilemmas for our distant descendants. Our role now ought to be keeping the ethical and societal issues engendered by GRIN technologies before our children and grandchildren so that they and their descendants will have the tools to make wise decisions about our species' future. One way of doing this is to formalize study of the science/technology – ethics interface in public education. In the U.S., government institutions have taken on this effort with programs like the ELSI (Ethical, Legal, and Social Implications) component of the Human Genome Project, funded by the Department of Energy and the National Institutes of Health, and with National Science Foundation Programs including Nanotechnology Undergraduate Education (NUE) and Ethics Education in Science and Engineering (EESE). Individual parents, teachers and State Academies of Science must commit similarly to self-education and teaching.

Index

Alabama Academy of Science Journal

Scope of the Journal:

The Alabama Academy of Science publishes significant, innovative research of interest to a wide audience of scientists in all areas. Papers should have a broad appeal, and particularly welcome will be studies that break new ground or advance our scientific understanding.

Information for the Authors:

- Manuscript layout should follow the specific guidelines of the journal.
- The authors are encouraged to contact the editor (E-mail: sah@jsu.edu) prior to paper submission to obtain the guidelines for the author.
- At least one author must be a member of the *Alabama Academy of Science* (except for Special Papers).
- The author(s) should provide the names and addresses of at least two potential reviewers.
- Assemble the manuscript in the following order: Title Page, Abstract Page, Text, Brief acknowledgments (if needed), Literature Cited, Figure Legends, Tables, Figures.

What and Where to Submit:

The original and two copies of the manuscript and a cover letter should be submitted to the following.

Dr. Safaa Al-Hamdani
Editor-Alabama Academy of Science Journal
Biology Department
Jacksonville State University
700 Pelham Road North
Jacksonville, AL 36265-1602

Review Procedure and Policy:

Manuscripts will be reviewed by experts in the research area. Manuscripts receiving favorable reviews will be tentatively accepted. Copies of the reviewers' comments (and reviewer-annotated files of the manuscript, if any) will be returned to the correspondent author for any necessary revisions. The final revision and electronic copy are then submitted to the *Alabama Academy of Science Journal* Editor. The author is required to pay $100 for partial coverage of printing costs of the article.